# On Etiology and Pathophysiology of Acute Pancreatitis : WITH SPECIAL REFERENCE TO PARTICIPATION OF PHOSPHOLIPASE A

AUTHOR(S):

HATAO, MASAHIKO

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# On Etiology and Pathophysiology of Acute Pancreatitis

## WITH SPECIAL REFERENCE TO PARTICIPATION OF PHOSPHOLIPASE A

by

MASAHIKO HATAO

From the 1st Division of Surgery Kyoto University, Medical School
(Director: Prof. Dr. ICHIO HONJO)

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## I. INTRODUCTION

Since KLEBS for the first time theoretically explained, almost a century ago, the role of pancreatic enzymes in acute pancreatitis, numerous studies have been carried out concerning the interrelationship between acute pancreatitis and pancreatic enzymes. Above

all, most intensively acting proteolytic enzymes, particularly trypsin, have been widely studied from various aspects, and it is generally accepted that trypsin plays an important role enzymochemically and pharmacodynamically in pathophysiology of acute pancreatitis. In 1934, however, DRAGSTEDT[20] was the first who doubted the essential etiologic significance in acute pancreatitis from the fact that trysin does not act upon lipoids and cellular membrane is composed of lipoid material. SUZUKI[87], BECK et al.[7][8], FUJIYAMA[29] and HAVERBACK et al.[44] failed to demonstrate trypsin activity in biliary pancreatitis the former two in the pancreatic tissue and the latter two in pancreatic juice, and it was furthermore pointed out that experimentally produced biliary pancreatitis is morphologically different from pancreatitis caused by trypsin[95]. ELMSLIE[25][26] also reported that pancreatitis could not be produced even if activated trypsin was infused into the pancreatic duct, unless the trypsin was infused with high pressure. Thus, there are not a few opinions against the concept in which particular emphasis is put on trypsin in the cause of acute pancreatitis.

Trypsin is essentially possessed of very little effect on protein of normally viable cells [1][19], and, for the manifestation of dynamic trypsin activity, it is indispensable that cells are already impaired in some way. As has been surveyed in the above, concerning the etiology and pathophysiology of acute pancreatitis, existence of some other factor which plays some role in earlier stage than trypsin acts, should be admitted.

ISHIKAWA[50] observed that in dogs with experimentally produced acute pancreatic necrosis tissue respiration of the liver gradually decreased with accompanying increase in activity of phospholipase C produced by Clostridium welchii within the liver, and this may be sometimes one of the lethal factors. He further observed intense activity of phospholipase in the pancreatic tissue or pancreatic juice, which cannot be neutralized, even in normal animals, by anti-$\alpha$-toxin. Since BÓKAY's report[10] in 1877, it has been widely known that similarly to the lethal toxin of phospholipase C, there exists phospholipase A in the pancreas, which acts upon phospholipid. Phospholipase A is contained in animal toxin like snake venom, and it has attracted interest from various aspects as a substance which reveals strong toxicity by itself or together with hemolytic effect of lysolecithin produced as a result of action on lecithin[4][11][12][15][23][34][38][39][64]. Membranous components of cells such as cellular membrane is mainly composed of phospholipid, and it is easily supposed that cells can not maintain normal function if the cellular membrane is affected by phospholipase.

In acute pancreatitis, various enzymes are activated or mobilized to the whole body with resulting various signs characterizing clinical symptoms of this disease. Phospholipase A, one of the pancreatic enzymes, has been considered to exist most abundantly in the pancreas[30] and to be possessed of strong toxicity. However, few studies have been made on the attitude and pathophysiologic role of phospholipase A in acute pancreatitis. In 1961, ZIEVE[98] observed an increase in phospholipase A in peripheral blood of patients with acute pancreatitis, and postulated diagnostic significance of determination of phospholipase A. CREUTZFELDT[13] also reported in 1966 that fulminant acute pancreatitis could be experimentally produced by infusion of lysolecithin or mixture of phospholipase A and cholic acid into the pancreatic duct, and he pointed out the important role of phospholipase A in etiology of acute pancreatitis. Concerning the role of phospholipase A in acute pancreatitis, however, only a few studies have been reproted.

Independently from these studies, in our clinic, originating from the study on phospholipase C at interruption of the hepatic artery[96], studies have been carried out on the significance of phospholipase C in acute pancreatic necrosis[50]. In the present experiment, it was the author's intention to investigate phospholipase A, one of the pancreatic enzymes, which is considered presumably to play an important role together with trypsin in etiology and pathophysiology of acute pancreatitis. In this aim, activity of phospholipase A and its fluctuation were investigated with the lapse of time in the pancreas, liver, portal blood, peripheral blood and peritoneal fluid of dogs with experimentally produced acute pancreatitis. Moreover, phospholipase A, extracted from the bovine pancreas, was infused into the portal vein or retroperitoneal space in order to investigate the influence of phospholipase A on organism. Studies were further carried out on the significance of phospholipase A in etiologic process of acute pancreatitis.

## II. MATERIALS AND METHODS

1. Experimental Animal

Healthy adult mongrel dogs of both sexes weighing from 6 to 15 kg were used.

2. Measurement of Arterial and Portal Pressures

For measurement of arterial pressure, the femoral artery was exposed, into which a flexible plastic canula was inserted being U-shaped mercurial manometer connected. Portal pressure was measured by aqueous manometer connected with a vinyl tube which was inserted into the portal vein through one of the branches of the superior mesenteric vein.

3. Extraction of Phospholipase A

Phospholipase A was extracted following the method of RIMON and SHAPIRO[79] from the bovine pancreas, which had been obtained immediately after slaughter and preserved for 2 weeks being frozen. Namely, frozen pancreas was warmed and homogenized by Waring's blender. The homogenate was filtered through double sheets of gauze. The filtrate (preparation I) was centrifuged at 3° to 6°C and 20,000 G for 1 hour, and the sediment was resuspended in 0.05 M-EDTA-2Na solution of pH 6.0. It was centrifuged again at 3° to 6°C and 20,000 G for 10 minutes. The sediment was resuspended in water and centrifuged. The sediment was resuspended in 0.067 M Soerensen phosphate buffer of pH 6.5 (preparation II). By these procedures, soluble proteins can be separated and removed. Then, preparation II was heated at 70° to 75°C for 10 minutes to destruct phospholipase B, and centrifuged at 3° to 6°C and 20,000 G for 15 minutes. The obtained sediment (preparation III) was dissolved in 0.1 M glycin-NaOH buffer of pH 9.8 (containing 32%v/v of ethanol) at room temperature and centrifuged at 3° to 6°C and 13,000 G for 10 minutes to remove unsoluble substances. The supernatant was adjusted to pH 6.5 to 7.0 resulting in precipitation, which was centrifuged at 3° to 6°C and 20,000 G for 20 minutes to separate the sediment. The sediment was dissolved in 0.067 M phosphate buffer of pH 7.0 (preparation IV). The obtained preparation IV manifests decomposition capacity of ovolecithin of 0.0324 $\mu$ moles/mg min. in 2 : 4 : 6 collidine buffer at 30°C[57]. This preparation IV was used as phospholipase A in the present experiment. The protein content was determined following the method of LOWRY[56].

4. Production of Acute Pancreatitis in Animals

Dogs were anesthetized with intravenous administration of Nembutal of 25 mg/kg

body weight. The abdomen was opened by upper midline incision. The accessory pancreatic duct was ligated and the main pancreatic duct was exposed, into which pancreatitis producing agents were gently infused. Then the main pancreatic duct was doubly ligated and cut. As pancreatitis producing agent, 0.3 cc/kg body weight of autogenous bile was used for investigation of fluctuation in phospholipase A activity. At the same time, phospholipase A suspension of 10 mg/cc, extracted from bovine pancreas, was infused into the main pancreatic duct in various doses with sodium deoxycholate in order to explore the appearance of on-set of acute pancreatitis. For the control study, false laparotomy was performed and the pancreatic duct was exposed and bile was discarded.

5. Determination of Phospholipase A Activity

i. Purification of Substrate (Lecithin)

Ovolecithin was extracted and purified from fresh yolk following the method of PANGBORN[72] which was used for substrate. Yolk was treated with aceton and added with 95 per cent ethanol, which was then vigorously shaken and filtered by aspiration. Solution of $CdCl_2$ was added to the filtrate and precipitation of lecithin-$CdCl_2$ was purified with chloroform, ethanol and petroleum ether, which was further dissolved in chloroform and vigorously shaken with 30 per cent alcohol to dissociate lecithin from $CdCl_2$ compound. The precipitation was dried under decompression. The obtained lecithin was dissolved in anhydrous ether and filtered by BUCHNER's funnel. By this procedure, flocculent precipitate could be removed. Remaining clear filtrate was dried under vacuum. Thus, finally purified lecithin was obtained, which was stored in refrigerater being solved in a small amount of absolute alcohol until used. Quantitative determination of lecithin was done through determination of phosphorus[27].

ii. Sample (Source of Phospholipase A)

Pancreatic tissue, hepatic tissue, portal blood, peripheral blood and peritoneal fluid were collected from experimental animals before the production of acute pancreatitis, 6, 12, 18, 24, 48 and 72 hours after the production of acute pancreatitis, and phospholipase A activity was determined in each sample, regarding them as enzymatic source. Pancreatic sample was taken from the inferior arm of the pancreas in a small section and used in a 10 per cent homogenate. Liver sample was taken from the left inferior lobe and used in a 33 per cent homogenate. Portal blood was collected directly from the portal vein in the liver hilum, and peripheral blood was collected from the femoral vein, both of these being immediately used after separation of serum. Peritoneal fluid was collected directly around the duodenum.

iii. Incubation System

Following the method of ZIEVE[98], enzyme source of 1.0 cc was added to 0.05 M borate buffer of 1.0 cc with pH 8.45, which was preheated for 30 minutes at 55°C. The mixture was added to 30 $\mu$M of substrate (ovolecithin) and 20 mg of deoxycholate suspended in 4 cc of 0.05 M borate buffer, and this was incubated for certain length of time at 55°C. Duration of the incubation differed depending on the source of enzyme, it being 60 minutes for pancreatic sample, 180 minutes for hepatic sample and peritoneal fluid and 18 hours for serum.

Phospholipase A activity was determined from the decrease in acyl ester bond which is produced as a result of the process of incubation in which substrate lecithin is decomposed

and converted to lysolecithin.

iv. Acyl Ester Content

Acyl ester content was determined following the hydroxamic acid method of STERN and SHAPIRO[86].

v. Unit

An amount of phospholipase A which decomposes 0.0001 $\mu$M of substrate per 1 minute was expressed as 1 unit.

## III. RESULTS

### A. Changes of Phospholipse A Activity in Dogs with Acute Biliary Pancreatitis

**Tab. 1.** Phospholipase A Activity (units/g) within Pancreas

Dogs with Acute Pancreatitis

| Dog No. | Sex | Body Weight (kg) | Before Infusion | After Infusion (hours) | | | | | | Survival Time (hours) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 6 | 12 | 18 | 24 | 48 | 72 | |
| 6 | ♂ | 14.0 | 5200 | 13600 | | | | | | 6 |
| 7 | ♂ | 14.5 | — | 10800 | 22000 | | | | | 14 |
| 8 | ♂ | 10.0 | 5600 | 14000 | 2800 | | | | | 17 |
| 9 | ♀ | 12.0 | 5600 | 22800 | 30400 | | | | | 13 |
| 10 | ♀ | 12.0 | 2800 | 5600 | 5600 | | | | | 16 |
| 11 | ♂ | 14.0 | 8400 | 8400 | 17600 | 21600 | 29600 | | | 24 |
| 12 | ♂ | 8.5 | 8400 | 5600 | 15000 | | | | | 12 |
| 13 | ♂ | 7.5 | 2800 | 2800 | 5600 | — | 14400 | 11600 | 11600 | 72 |
| 14 | ♂ | 11.0 | 18400 | 16400 | 21600 | 24800 | 22400 | | | 28 |
| 15 | ♂ | 14.5 | 5600 | 13600 | — | 30400 | | | | 20 |
| 16 | ♂ | 10.0 | 2800 | 8800 | 13200 | 17200 | 22400 | 19600 | 22400 | 72 |
| 17 | ♂ | 14.0 | 2800 | 5600 | 5600 | 5200 | | | | 20 |
| 18 | ♂ | 10.0 | 5600 | 11600 | 17200 | 19600 | 22400 | | | 30 |
| 19 | ♂ | 9.0 | 8400 | 11200 | 14000 | 22400 | 22400 | | | 39 |
| 20 | ♂ | 8.5 | 11200 | 16800 | 19600 | | | | | 13 |
| 21 | ♂ | 7.2 | 8400 | — | 14000 | — | 22400 | 22400 | | 50 |
| 22 | ♂ | 7.5 | 11600 | — | 10800 | — | 16000 | 14000 | 8400 | 72 |
| 23 | ♂ | 9.5 | 8400 | — | 8400 | — | 11200 | 8400 | 11200 | Survived |
| 24 | ♂ | 7.5 | 8400 | — | 14000 | — | 22400 | 19600 | | 48 |
| 25 | ♂ | 9.5 | 8400 | — | 25200 | | | | | 12 |
| 26 | ♀ | 7.5 | 8400 | — | 11200 | — | 22400 | 16800 | | 52 |
| 27 | ♂ | 10.0 | 11200 | — | 28000 | | | | | 14 |
| Mean | | | 7500 | 11200 | 15000 | 20200 | 20700 | 16100 | 13400 | |

Control Animals

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ♀ | 12.0 | 5200 | 8400 | 8400 | — | 11200 | 11200 | | Survived |
| 2 | ♂ | 8.0 | 8400 | 3200 | 8800 | 8000 | — | 2800 | 8000 | Survived |
| 3 | ♂ | 7.0 | 8400 | 11200 | 5600 | 5600 | 8400 | 5600 | 5600 | Survived |
| Mean | | | 7300 | 7600 | 7600 | 6800 | 9800 | 6500 | 6800 | |

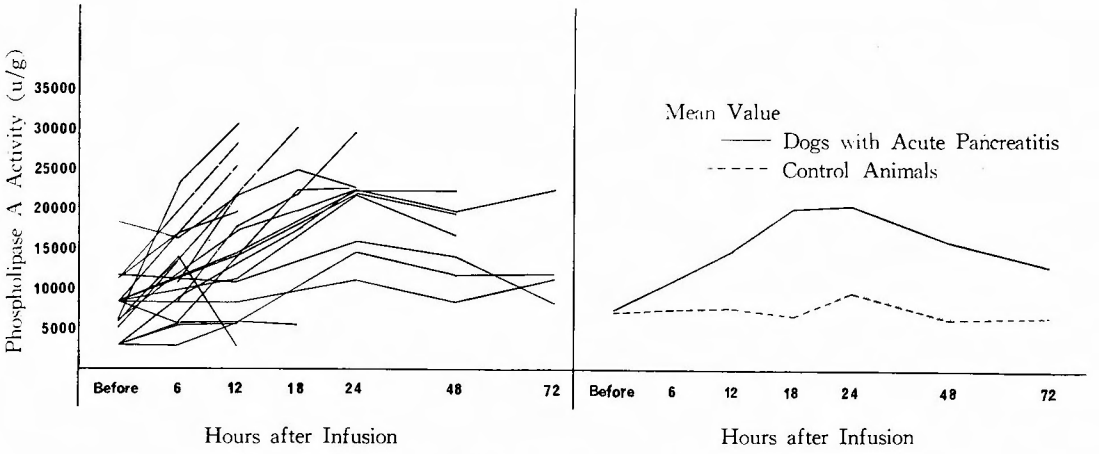


**Fig. 1.** Fluctuation in Phospholipase A Activity within Pancreas

1. Changes of Phospholipase A Activity within Pancreas

Phospholipase A activity within the pancreas before the production of acute pancreatitis ranged from 2, 800 to 18, 400 units/g, 7, 500 units/g on the average, as shown in Tab. 1. Phospholipase A activity increased from 6 hours after the production of acute pancreatitis, reaching the highest level from 18 to 24 hours after the production of acute pancreatitis, then showing a tendency of gradual decrease or maintenance in a higher level, as shown in Tab. 1 and Fig. 1. The average activity of 21 lethal cases was as 2. 5 times higher than preoperative level, as determined immediately befor death. In 3 cases of control study, little fluctuation could be observed in phospholipase A activity within the pancreas, as in Tab. 1.

2. Changes of Phospholipase A Activity within Liver

Phospholipase A activity within the liver ranged from 0 to 1, 050 units/g, 520 units/g on the average, before the production of acute pancreatitis, as shown in Tab. 2. After the production of acute pancreatitis, phospholipase A activity increased also in the liver with a tendency of gradual increase with the lapse of time. In lethal cases, decrease of the activity could not be observed, as shown in Tab. 2 and Fig. 2, and the average activity as determined immediately before death was as 3 times higher than preoperative level. In cases of false operation, only slight increase in phospholipase A activity could be observed in the liver after surgery, and there was no lethal case.

3. Changes of Phospholipase A Activity in Portal Blood

Phospholipase A activity in portal blood ranged from 6. 7 to 31. 1 units/cc, 17. 1 units/cc on the average before surgery, as summarized in Tab. 3. After surgery, phospholipase A activity remarkably increased on one way, and the average activity was as 6. 1 times higher than preoperative level as determined immediately before death, as shown in Tab. 3 and Fig. 3. There was no case which showed the decrease in the activity. In cases of false operation, little fluctuation could be observed in phospholipase A activity.

4. Changes of Phosholipase A Activity in Peripheral Blood

Before the production of acute pancreatitis, phospholipase A activity in peripheral blood ranged from 0 to 23. 3 units/cc, 14. 0 units/cc on the average, as in Tab. 4. Phospholi-

**Tab. 2.** Phospholipase A Activity (units/g) within Liver

| Dog. No. | Sex | Body Weight (kg) | Before Infusion | After Infusion (hours) 6 | 12 | 18 | 24 | 48 | 72 | Survival Time (hours) |
|---|---|---|---|---|---|---|---|---|---|---|
| Dogs with Acute Pancreatitis | | | | | | | | | | |
| 6 | ♂ | 14.0 | 700 | 1100 | | | | | | 6 |
| 7 | ♂ | 14.5 | 350 | — | 700 | | | | | 14 |
| 8 | ♂ | 10.0 | 700 | 300 | 300 | | | | | 17 |
| 9 | ♀ | 12.0 | 0 | 1400 | 2100 | | | | | 13 |
| 10 | ♀ | 12.0 | 350 | 700 | 1050 | | | | | 16 |
| 11 | ♂ | 14.0 | 350 | 0 | 800 | 1100 | 2100 | | | 24 |
| 12 | ♂ | 8.5 | 700 | 700 | 1050 | | | | | 12 |
| 13 | ♂ | 7.5 | 700 | 300 | 350 | 1100 | 750 | 1000 | 1450 | 72 |
| 14 | ♂ | 11.0 | 0 | 650 | 650 | 700 | 1050 | | | 28 |
| 15 | ♂ | 14.5 | 350 | 350 | 1150 | 1100 | | | | 20 |
| 16 | ♂ | 10.0 | 750 | 1050 | 1100 | — | 1100 | 1500 | 1850 | 72 |
| 17 | ♂ | 14.0 | 0 | — | 750 | 700 | | | | 20 |
| 18 | ♂ | 10.0 | 700 | 1050 | 1050 | 1750 | 2450 | | | 30 |
| 19 | ♂ | 9.0 | 700 | 1050 | 1050 | 1400 | 1750 | | | 39 |
| 20 | ♂ | 8.5 | 700 | 1400 | 2450 | | | | | 13 |
| 21 | ♂ | 7.2 | 350 | — | 700 | — | 1050 | 1750 | 1350 | 50 |
| 22 | ♂ | 7.5 | 350 | — | 0 | — | 700 | 700 | 350 | 72 |
| 23 | ♂ | 9.5 | — | — | 700 | — | 1050 | — | | Survived |
| 24 | ♂ | 7.5 | 700 | — | 700 | — | 1050 | 1400 | | 48 |
| 25 | ♂ | 9.5 | 700 | — | 2450 | | | | | 12 |
| 26 | ♀ | 7.5 | 700 | — | 1050 | — | 1050 | 1400 | | 52 |
| 27 | ♂ | 10.0 | 1050 | — | 2800 | | | | | 14 |
| Mean | | | 520 | 780 | 1090 | 1120 | 1280 | 1290 | 1250 | |
| Control Animals | | | | | | | | | | |
| 1 | ♀ | 12.0 | 650 | 350 | 0 | 700 | 1350 | 350 | — | Survived |
| 2 | ♂ | 8.0 | 0 | 650 | 350 | 1100 | 0 | 700 | 350 | Survived |
| 3 | ♂ | 7.0 | 300 | 0 | 700 | 700 | — | 350 | — | Survived |
| Mean | | | 320 | 330 | 350 | 830 | 680 | 470 | 350 | |

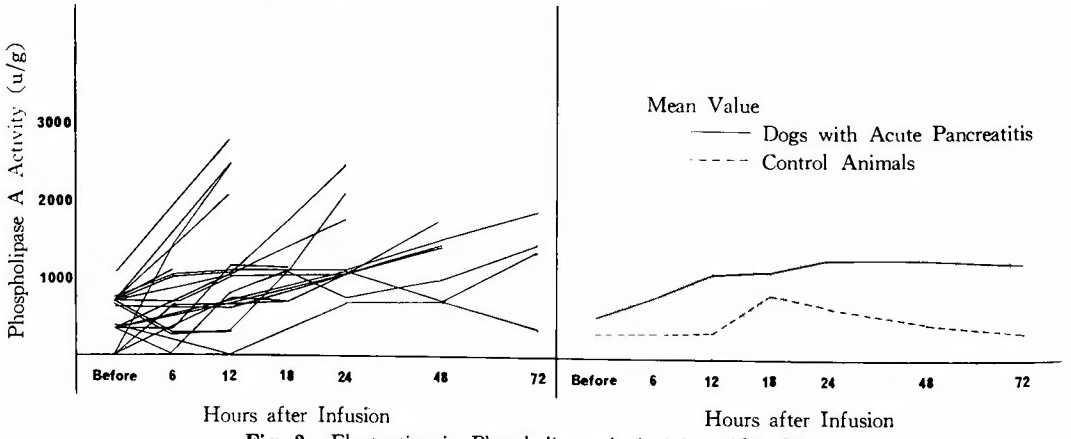


**Fig. 2.** Fluctuation in Phospholipase A Activity within Liver

**Tab. 3.** Phospholipase A Activity (units/cc) in Portal Blood

| Dog No. | Sex | Body Weight (kg) | Before Infusion | After Infusion (hours) | | | | | | Survival Time (hours) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 6 | 12 | 18 | 24 | 48 | 72 | |
| Dogs with Acute Pancreatitis | | | | | | | | | | |
| 6 | ♂ | 14.0 | 7.8 | 40.0 | | | | | | 6 |
| 7 | ♂ | 14.5 | 6.7 | — | 146.7 | | | | | 14 |
| 8 | ♂ | 10.0 | 7.8 | 0 | 38.9 | | | | | 17 |
| 9 | ♀ | 12.0 | 14.4 | 37.8 | 52.2 | | | | | 13 |
| 10 | ♀ | 12.0 | 14.4 | 54.4 | 63.3 | | | | | 16 |
| 11 | ♂ | 14.0 | 23.3 | 47.8 | 101.1 | 108.8 | 255.5 | | | 24 |
| 12 | ♂ | 8.5 | 22.2 | 16.7 | 63.3 | | | | | 12 |
| 13 | ♂ | 7.5 | 23.3 | 22.2 | 33.3 | 7.8 | 46.7 | 61.1 | 68.9 | 72 |
| 14 | ♂ | 11.0 | 7.8 | 63.3 | 85.5 | 86.6 | 100.0 | | | 28 |
| 15 | ♂ | 14.5 | 7.8 | 53.3 | 75.6 | 115.6 | | | | 20 |
| 16 | ♂ | 10.0 | 24.4 | 38.9 | 53.3 | 53.3 | 38.9 | 76.7 | 94.4 | 72 |
| 17 | ♂ | 14.0 | 15.6 | 46.7 | 70.0 | 70.0 | | | | 20 |
| 18 | ♂ | 10.0 | 15.6 | 38.9 | 46.7 | 85.6 | 131.1 | | | 30 |
| 19 | ♂ | 9.0 | 23.3 | 54.4 | 62.2 | 92.2 | 108.9 | | | 39 |
| 20 | ♂ | 8.5 | 23.3 | 62.2 | 155.6 | | | | | 13 |
| 21 | ♂ | 7.2 | 31.1 | — | 38.9 | — | 77.8 | 101.1 | | 50 |
| 22 | ♂ | 7.5 | 22.2 | — | 38.9 | — | — | 46.7 | 55.6 | 72 |
| 23 | ♂ | 9.5 | 15.6 | — | 32.2 | — | 40.0 | 7.8 | 46.7 | Survived |
| 24 | ♂ | 7.5 | 7.8 | — | 23.3 | — | 77.8 | 98.9 | | 48 |
| 25 | ♂ | 9.5 | 23.3 | — | 185.6 | | | | | 12 |
| 26 | ♀ | 7.5 | 15.6 | — | 38.9 | — | 62.2 | 92.2 | | 52 |
| 27 | ♂ | 10.0 | 23.3 | — | 168.9 | | | | | 14 |
| Mean | | | 17.1 | 41.2 | 75.0 | 77.5 | 93.9 | 69.2 | 66.4 | |
| Control Animals | | | | | | | | | | |
| 1 | ♀ | 12.0 | 7.8 | — | 15.6 | 14.4 | 22.2 | 7.8 | 15.6 | Survived |
| 2 | ♂ | 8.0 | 23.3 | 22.2 | 16.7 | 0 | 22.2 | 6.7 | 0 | Survived |
| 3 | ♂ | 7.0 | 14.4 | 22.2 | 15.6 | 22.2 | 7.8 | 15.6 | 23.3 | Survived |
| Mean | | | 15.2 | 22.2 | 14.3 | 12.2 | 17.4 | 10.0 | 13.0 | |

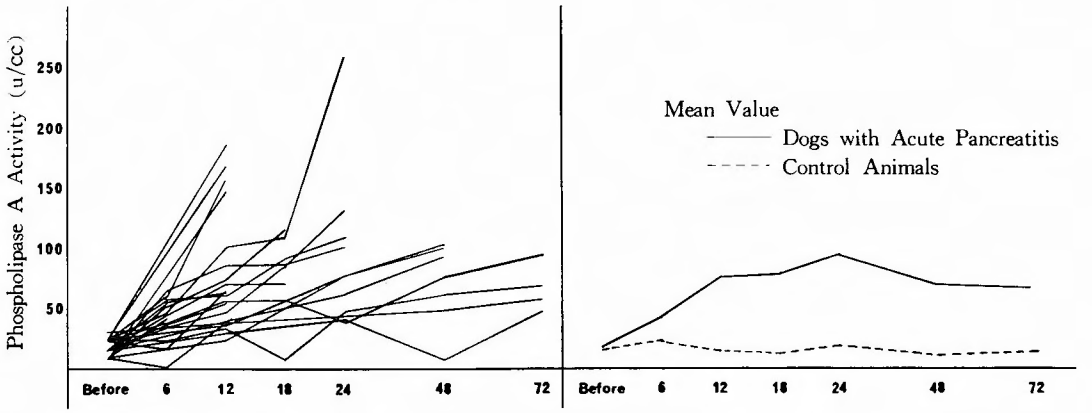


**Fig. 3.** Fluctuation in Phospholipase A Activity in Portal Blood

**Tab. 4.** Phospholipase A Activity (units/cc) in Peripheral Blood
Dogs with Acute Pancreatitis

| Dog No. | Sex | Body Weight (kg) | Before Infusion | After Infusion (hours) 6 | 12 | 18 | 24 | 48 | 72 | Survival Time (hours) |
|---|---|---|---|---|---|---|---|---|---|---|
| 6 | ♂ | 14.0 | 15.6 | 24.4 | | | | | | 6 |
| 7 | ♂ | 14.5 | 0 | — | 125.5 | | | | | 14 |
| 8 | ♂ | 10.0 | 16.7 | 32.2 | 24.4 | | | | | 17 |
| 9 | ♀ | 12.0 | 14.4 | 30.0 | 37.8 | | | | | 13 |
| 10 | ♀ | 12.0 | 7.8 | 31.1 | 31.1 | | | | | 16 |
| 11 | ♂ | 14.0 | 7.8 | 24.4 | 54.4 | 62.2 | 92.2 | | | 24 |
| 12 | ♂ | 8.5 | 14.4 | 7.8 | 23.3 | | | | | 12 |
| 13 | ♂ | 7.5 | 14.4 | — | 15.6 | 31.1 | 38.9 | 46.7 | 54.4 | 72 |
| 14 | ♂ | 11.0 | 16.7 | 21.1 | 48.8 | 45.6 | 46.7 | | | 28 |
| 15 | ♂ | 14.5 | 15.6 | 38.9 | 62.2 | 6191 | | | | 20 |
| 16 | ♂ | 10.0 | 23.3 | 23.3 | 46.7 | 31.1 | 45.6 | 38.9 | 45.6 | 72 |
| 17 | ♂ | 14.0 | 14.4 | 24.4 | 14.4 | 23.3 | | | | 20 |
| 18 | ♂ | 10.0 | 15.6 | 23.3 | 31.1 | 46.7 | 62.2 | | | 30 |
| 19 | ♂ | 9.0 | 15.6 | 31.1 | 38.9 | 62.2 | 70.0 | | | 39 |
| 20 | ♂ | 8.5 | 7.8 | 38.9 | 62.2 | | | | | 13 |
| 21 | ♂ | 7.2 | 15.6 | — | 31.1 | — | 46.7 | 54.4 | | 50 |
| 22 | ♂ | 7.5 | — | — | 33.3 | — | 23.3 | 15.6 | 38.9 | 72 |
| 23 | ♂ | 9.5 | 16.7 | — | 16.7 | — | 31.1 | 23.3 | 7.8 | Survived |
| 24 | ♂ | 7.5 | 7.8 | — | 15.6 | — | 46.7 | 70.0 | | 48 |
| 25 | ♂ | 9.5 | 23.3 | — | 62.2 | | | | | 12 |
| 26 | ♀ | 7.5 | 15.6 | — | 31.1 | — | 46.7 | 70.0 | | 52 |
| 27 | ♂ | 10.0 | 15.7 | — | 77.8 | | | | | 14 |
| Mean | | | 14.0 | 27.0 | 41.6 | 45.4 | 50.0 | 45.6 | 36.7 | |

Control Animals

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ♀ | 12.0 | — | 14.4 | 7.8 | — | 22.2 | 11.4 | 14.4 | Survived |
| 2 | ♂ | 8.0 | 7.8 | — | 24.4 | 7.8 | — | 14.4 | 0 | Survived |
| 3 | ♂ | 7.0 | 15.6 | 15.6 | 7.8 | 30.0 | 22.2 | 6.7 | 16.7 | Survived |
| Mean | | | 11.4 | 15.0 | 13.3 | 18.9 | 22.2 | 11.8 | 10.4 | |

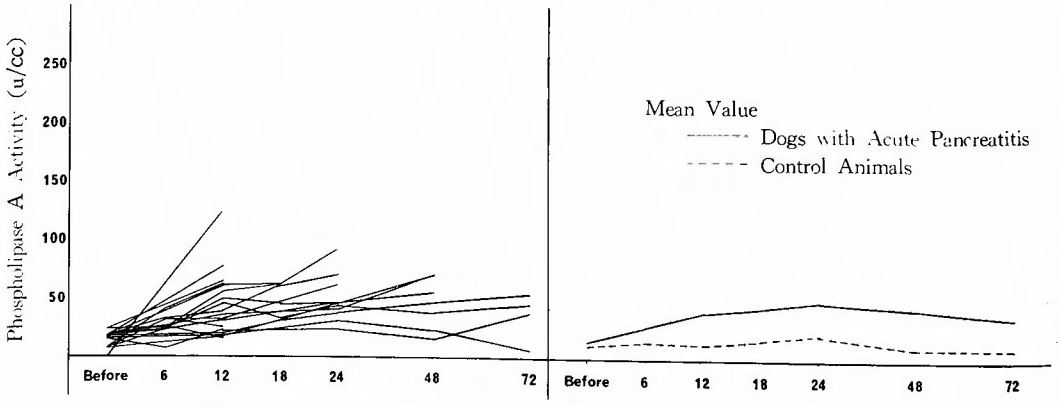


**Fig. 4.** Fluctuati on in Phospholipase A Activity in Peripheral Blood

**Tab. 5.** Phospholipase A Activity (units/cc) in Peritoneal Fluid Dogs with Acute Pancreatitis

| Dog No. | Sex | Body Weight (kg) | After Infusion (hours) | | | | | | Survival Time (hours) |
|---|---|---|---|---|---|---|---|---|---|
| | | | 6 | 12 | 18 | 24 | 48 | 72 | |
| 6 | ♂ | 14.0 | 420 | | | | | | 6 |
| 7 | ♂ | 14.5 | 280 | 420 | | | | | 14 |
| 8 | ♂ | 10.0 | 280 | 140 | | | | | 17 |
| 9 | ♀ | 12.0 | 280 | 420 | | | | | 13 |
| 10 | ♀ | 12.0 | 140 | 120 | | | | | 16 |
| 11 | ♂ | 14.0 | 280 | 440 | 560 | 580 | | | 24 |
| 12 | ♂ | 8.5 | 280 | 580 | | | | | 12 |
| 13 | ♂ | 7.5 | 140 | 280 | 280 | 420 | 560 | 720 | 72 |
| 14 | ♂ | 11.0 | 260 | 560 | 540 | 680 | | | 28 |
| 15 | ♂ | 14.5 | 140 | 280 | 420 | | | | 20 |
| 16 | ♂ | 10.0 | 280 | 580 | 540 | 840 | 560 | 680 | 72 |
| 17 | ♂ | 14.0 | 140 | 140 | 260 | | | | 20 |
| 18 | ♂ | 10.0 | 420 | 700 | 840 | 840 | | | 30 |
| 19 | ♂ | 9.0 | 280 | 420 | 420 | 560 | | | 39 |
| 20 | ♂ | 8.5 | 420 | 700 | | | | | 13 |
| 21 | ♂ | 7.2 | — | 420 | — | 560 | 560 | | 50 |
| 22 | ♂ | 7.5 | — | 420 | — | 1000 | 700 | 1000 | 72 |
| 23 | ♂ | 9.5 | — | 420 | — | 280 | 540 | 680 | Survived |
| 24 | ♂ | 7.5 | — | 280 | — | 560 | 560 | | 48 |
| 25 | ♂ | 9.5 | — | 980 | | | | | 12 |
| 26 | ♀ | 7.5 | — | 280 | — | 560 | 700 | | 52 |
| 27 | ♂ | 10.0 | — | 1120 | | | | | 14 |
| Mean | | | 270 | 460 | 480 | 630 | 600 | 770 | |

pase A activity increased after the production of acute pancreatitis, and the average activity was as 3.9 times higher than preoperative level, as determined immediately before death, as shown in Tab. 4 and Fig. 4. In control animals, only slight fluctuation could be observed in phospholipase A activity.

5. Changes of Phospholipase A Activity in Peritoneal Fluid

Before the production of acute pancreatitis, adequate amount of fluid for determination of phospholipase A activity could not be found in the peritoneal cavity of any experimental animals, whereas after the production of acute pancreatitis bloody fluid accumulation could be observed in the peritoneal cavity of all experimental animals. Phospholipase A activity in peritoneal fluid 6 hours after the pro-

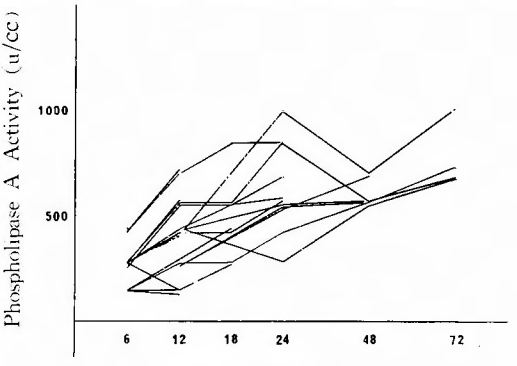


**Fig. 5.** Fluctuation in Phospholipase A Activity in Peritoneal Fluid

duction of acute pancreatitis was 270 units/cc on the average, which was obviously higher than in serum, and it further increased on to the average activity of 600 units/cc as determined immediately before death, as shown in Tab. 5 and Fig. 5.

6. Summary

In the pancreatic tissue of normal dogs, markedly higher activity of phospholipase A could be observed than in hepatic tissue or in serum. This activity of phospholipase A further increased at acute biliary pancreatitis, such a tendency being particularly pronounced approximately in two thirds of the experimental animals. In parallel with fluctuation of phospholipase A activity within the pancreas, phospholipase A activity also increased in portal blood, hepatic tissue, peripheral blood and peritoneal fluid in dogs with acute pancreatitis, the extent of the increase, however, being rather outstanding in these samples, particularly in portal blood, than in the pancreatic tissue.

Concerning the relationship between the fluctuation in phospholipase A activity and survival time of the experimental dogs, there was a tendency that the earlier and the more intense the increase in phospholipase A activity appeared after the production of acute pancreatitis, the earlier the experimental animals died. Particularly, it was considered that the increase in phospholipase A activity in portal blood and the liver has a large influence on survival time of the animals. In this respect, an analysis was made, 14 experimental dogs with particularly intense increase in phospholipase A activity (No. 7, 9, 11, 14, 15, 16, 18, 19, 20, 21, 24, 25, 26 and 27) being divided into two groups of early death, in which the animals died within 24 hours after surgery, and late death, in which the animals died later than 24 hours after surgery. As shown in Tab. 6, phospholipase A activity in portal blood in the group of remote death showed marked increase of 4.9 times of preoperative level, similarly the activity in the liver being 2.5 time of preoperative level. In contrast to the findings, in the group of early death, the increase in phospholipase A activity was more intense to be 8.4 times in portal blood and 4 times in the liver, as compared with preoperative level.

**Tab. 6.** Relationship between Increase in Phospholipase A Activity and Survival Time
(Summary of 14 cases with remarkable increase in phospholipase A activity)

| | Animals died within 24 Hours | | | Animals died Later than 24 Hours | | |
|---|---|---|---|---|---|---|
| | Level before Infusion (units) | Level Immediately before Death (units) | Degree of Increase (times compared with level before infusion) | Level before Infusion (units) | Level Immediately before Death (units) | Degree of Increase (times compared with level before infusion) |
| Pancreas | 9200 | 25200 | 2.7 | 7000 | 20300 | 2.9 |
| Liver | 510* | 2030 | 4.0 | 630 | 1600 | 2.5 |
| Portal Blood | 16.9 | 142.0 | 8.4 | 19.7 | 96.7 | 4.9 |
| Peripheral Blood | 13.3 | 69.8 | 5.2 | 15.6 | 60.0 | 3.8 |
| Peritoneal Fluid | — | 670 | — | — | 630 | — |

(* Dog No. 7 excluded)

B. Influence of Phospolipase A on Organism

1. Influence of Phospholipase A Administered into Femoral Vein in Dogs

Under intravenous anesthesia with Nembutal in dogs, 10 mg of phospholipase A (protein equivalent), extracted from bovine pancreas, per 1 kg of body weight was injected into the femoral vein together with 25 mg of sodium deoxycholate spending 3 minutes, and fluctuations in arterial and portal pressures were investigated.

Arterial pressure showed rapid decrease already before the ending of the injection of phospholipase A, decreasing by 55 to 62 mmHg, and reached 60 to 65 mmHg when the injection ended. This decrease in arterial pressure was temporary without falling into shock state, and the pressure increased shortly after the injection, restoring almost to normal level from 15 to 20 minutes later. However, the pressure did not return to the level before the injection, as investigated for 120 minutes. Portal pressure decreased slightly only by 6 to 7 $mmH_2O$ even 5 minutes after the commencement of the injection, in which period arterial pressure remarkably decreased. As shown in Tab. 7, portal pressure showed little fluctuation during the investigation for 120 minutes. Experimental animals soon awoke from the anesthesia, gradually doing well. On the next day, no abnormality could be observed in these animals, with favorable appetite. For the control study, 25 mg of sodium deoxy-

**Tab. 7.** Arterial and Portal Pressures after Infusion of Phospholipase A into Femoral Vein

| Time (min) | Arterial Pressure | | | | Portal Pressure | | | |
|---|---|---|---|---|---|---|---|---|
| | Exper. Animals | | Control Animals | | Exper. Animals | | Control Animals | |
| | No. 41 | No. 42 | No. 43 | No. 44 | No. 41 | No. 42 | No. 43 | No. 44 |
| Before | 122 | 120 | 132 | 118 | 102 | 110 | 120 | 95 |
| 3 | 60 | 65 | | | | | | |
| 5 | 86 | 72 | 130 | 124 | 95 | 104 | 122 | 92 |
| 10 | 88 | 80 | 130 | 124 | 100 | 104 | 120 | 95 |
| 15 | 96 | 96 | 128 | 124 | 106 | 100 | 118 | 100 |
| 20 | 102 | 96 | 130 | 128 | 106 | 102 | 118 | 102 |
| 25 | 100 | 96 | 132 | 124 | 106 | 104 | 118 | 104 |
| 30 | 100 | 100 | 132 | 124 | 110 | 104 | 118 | 104 |
| 35 | 102 | 95 | 132 | 124 | 112 | 104 | 118 | 100 |
| 40 | 100 | 100 | 132 | 124 | 110 | 104 | 118 | 96 |
| 45 | 98 | 102 | 130 | 126 | 108 | 104 | 118 | 100 |
| 50 | 102 | 104 | 132 | 124 | 110 | 102 | 116 | 96 |
| 55 | 104 | 106 | 128 | 124 | 108 | 104 | 116 | 95 |
| 60 | 106 | 104 | 130 | 122 | 102 | 102 | 116 | 95 |
| 70 | 106 | 106 | 130 | 124 | 100 | 102 | 118 | 98 |
| 80 | 106 | 106 | 130 | 124 | 100 | 102 | 114 | 95 |
| 90 | 106 | 105 | 130 | 124 | 100 | 100 | 118 | 95 |
| 100 | 106 | 104 | 128 | 122 | 100 | 102 | 118 | 92 |
| 110 | 106 | 104 | 130 | 124 | 102 | 102 | 116 | 92 |
| 120 | 106 | 104 | 130 | 122 | 100 | 104 | 118 | 95 |
| Survival Time | Survived | Survived | Survived | Survived | Survived | Survived | Survived | Survived |

(In dogs No. 43 and 44, for control study, sodium deoxycholate of 25 mg alone was infused.)

cholate was injected into the femoral vein, which resulted in little fluctuation in arterial and portal pressures, animals surviving.

2. Influence of Phospholipase A Administered into Portal Vein

Ten mg/kg body weight of phospholipase A was injected into the portal vein through a vinyl tube inserted into a branch of the superior mesenteric vein, spending 3 minutes, in anesthetized dogs as described in the above, and fluctuations in arterial and portal pressures were investigated.

In one case (No. 46), arterial pressure began to decrease during the injection, showing a decrease of 54 mmHg 45 minutes later. It gradually increased, however, without falling into shock state, and restored almost to normal range, though it was lower than the level before the injection, 120 minutes later. Portal pressure showed an increase of 80 $mmH_2O$ 5 minutes after the injection, which was followed by gradually decrease, restoring to normal level 120 minutes after the injection, as shown in Tab. 8. In another case (No. 47), arterial pressure rapidly decreased during the injection similarly to the cases of the injection from the femoral vein, and it decreased by 60 mmHg, when the injection ended. This was also temporary and restored to the preinjection level 120 minutes after

**Tab. 8.** Arterial and Portal Pressures after Infusion of Phospholipase A into Portal Vein

| Time (min) | Arterial Pressure | | | | | Portal Pressure | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Exper. Animals | | Control Animals | | | Exper. Animals | | Control Animals | | |
| | No. 46 | No. 47 | No. 48 | No. 49 | No. 50 | No. 46 | No. 47 | No. 48 | No. 49 | No. 50 |
| Before | 150 | 110 | 120 | 130 | 124 | 100 | 90 | 120 | 112 | 106 |
| 3 | 110 | 50 | | | | | | | | |
| 5 | 110 | 55 | 125 | 136 | 126 | 180 | 106 | 125 | 128 | 108 |
| 10 | 110 | 80 | 130 | 142 | 124 | 175 | 88 | 125 | 120 | 110 |
| 15 | 107 | 96 | 128 | 154 | 124 | 165 | 84 | 122 | 116 | 108 |
| 20 | 105 | 95 | 126 | 158 | 128 | 145 | 92 | 124 | 112 | 112 |
| 25 | 106 | 98 | 128 | 156 | 126 | 125 | 88 | 125 | 108 | 110 |
| 30 | 105 | 100 | 132 | 158 | 126 | 120 | 80 | 124 | 112 | 110 |
| 35 | 105 | 106 | 132 | 158 | 126 | 125 | 93 | 126 | 110 | 108 |
| 40 | 100 | 106 | 130 | 158 | 130 | 120 | 90 | 124 | 112 | 106 |
| 45 | 96 | 110 | 132 | 154 | 132 | 125 | 90 | 125 | 114 | 104 |
| 50 | 100 | 108 | 134 | 156 | 134 | 122 | 90 | 126 | 114 | 104 |
| 55 | 110 | 108 | 132 | 156 | 132 | 118 | 90 | 125 | 115 | 104 |
| 60 | 108 | 104 | 132 | 154 | 132 | 115 | 92 | 125 | 118 | 106 |
| 70 | 110 | 108 | 135 | 156 | 134 | 106 | 96 | 122 | 120 | 106 |
| 80 | 114 | 108 | 132 | 154 | 130 | 110 | 96 | 124 | 120 | 104 |
| 90 | 112 | 110 | 132 | 150 | 132 | 110 | 94 | 122 | 120 | 108 |
| 100 | 110 | 106 | 130 | 152 | 132 | 102 | 96 | 122 | 118 | 104 |
| 110 | 116 | 108 | 128 | 150 | 134 | 104 | 92 | 124 | 118 | 106 |
| 120 | 118 | 108 | 130 | 150 | 132 | 105 | 92 | 122 | 120 | 106 |
| Survival Time | 13 hrs. | 21 hrs. | Survived | Survived | Survived | 18 hrs. | 21 hrs. | Survived | Survived | Survived |

(In control dogs No. 48 and 50, sodium deoxycholate of 25 mg alone was infused, and in dog No. 49, bovine serum was infused in a dosis of 10 mg/kg with 25 mg of sodium deoxycholate.)

the injection without falling into shock state. Portal pressure showed little fluctution, as shown in Tab. 8. though with a slight increase of 16 mm$H_2O$ 5 minutes after the injection.

All the experimental animals looked to be exhausted and could not stand on the foot, showing no appetite after awoke from anesthesia. These animals died about 20 hours after the injection, as shown in Tab. 11.

For the control study, 25 mg of sodium deoxycholate was injected into the portal vein, and little fluctuation was observed in arterial and portal pressures. When 10 mg/kg body weight of bovine serum was injected into the portal vein with 25 mg of sodium deoxycholate, arterial pressure increased. However, control animals all survived, as shown in Tab. 8.

3. Influence of Phospholipase A Subcutaneously Administered

Ten mg of phospholipase A and 10 mg of sodium deoxycholate were subcutaneously administered in the abdominal side of intravenously anesthetized dogs, and arterial and portal pressures were investigated. There was little fluctuation in arterial and portal pressures, as shown in Tab. 9.

For the control study, 10 mg of sodium deoxycholate was similarly injected, and little fluctuation could be observed in arterial and portal pressures, all the animals surviving well, as shown in Tab. 9.

**Tab. 9.** Arterial and Portal Pressures after Subcutaneous Infusion of Phospholipase A

| Time (min) | Arterial Pressure | | | | Portal Pressure | | | |
|---|---|---|---|---|---|---|---|---|
| | Exper. Animals | | Control Animals | | Exper. Animals | | Control Animals | |
| | No. 51 | No. 52 | No. 53 | No. 54 | No. 51 | No. 52 | No, 53 | No. 54 |
| Before | 100 | 118 | 120 | 120 | 90 | 110 | 100 | 120 |
| 5 | 96 | 118 | 122 | 120 | 94 | 112 | 108 | 118 |
| 10 | 100 | 118 | 122 | 120 | 96 | 110 | 106 | 120 |
| 15 | 102 | 116 | 120 | 120 | 96 | 110 | 108 | 116 |
| 20 | 98 | 116 | 122 | 122 | 100 | 110 | 108 | 116 |
| 25 | 100 | 118 | 122 | 120 | 98 | 110 | 108 | 114 |
| 30 | 98 | 120 | 120 | 118 | 98 | 108 | 106 | 114 |
| 35 | 100 | 122 | 124 | 120 | 98 | 110 | 108 | 112 |
| 40 | 102 | 118 | 122 | 120 | 94 | 110 | 106 | 108 |
| 45 | 102 | 118 | 122 | 118 | 96 | 112 | 106 | 108 |
| 50 | 102 | 118 | 122 | 120 | 94 | 112 | 106 | 110 |
| 55 | 106 | 118 | 122 | 120 | 96 | 112 | 106 | 110 |
| 60 | 106 | 120 | 120 | 120 | 96 | 112 | 106 | 108 |
| 70 | 104 | 120 | 120 | 120 | 96 | 110 | 106 | 108 |
| 80 | 106 | 120 | 122 | 120 | 94 | 112 | 104 | 108 |
| 90 | 108 | 120 | 122 | 120 | 96 | 112 | 104 | 110 |
| 100 | 106 | 122 | 122 | 120 | 96 | 114 | 104 | 110 |
| 110 | 108 | 120 | 122 | 120 | 94 | 114 | 104 | 110 |
| 120 | 106 | 120 | 122 | 120 | 94 | 114 | 106 | 108 |
| Survival Time | Survived | Survived | Survived | Survived | Survived | Survived | Survived | Survived |

(In control dogs No. 53 and 54, 10 mg of sodium deoxycholate alone was infused.)

4. Influence of Phospholipase A Infused into Retroperitoneal Space

Following the trypsin infusion experiment of NAKGAWA[59], 5 mg or 10 mg of phospholipase A was infused into the retroperitoneal space of anesthetized dogs, with respectively the same amount of sodium deoxycholate, and arterial and portal pressures were investigated.

Arterial pressure increased by 8 to 24 mmHg immediately after the infusion, but it decreased at once and in some cases it decreased by 30 to 40 mmHg 30 to 50 minutes after the infusion, which was, however, also transient and soon restored to normal level without falling into shock state as investigated for 120 minutes. Portal pressure increased by 17 to 30 mm$H_2O$ 5 minutes after the infusion, and soon returned to the level before the infusion with little fluctuation, as shown in Tab. 10. The experimental animals looked to be exhausted when awoke, and 2 cases out of 3 died about 20 hours after the infusion, except a single case which received retroperitoneal infusion of 10 mg of phospholipase A, as shown in Tab. 11.

For the control study, 5 mg or 25 mg of sodium deoxycholate or saline solution alone

**Tab. 10.** Arterial and Portal Pressures after Infusion of Phospholipase A into Retroperitoneal Spase

| | Arterial Pressure | | | | | | | Portal Pressure | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time (min) | Exper. Animals | | | Control Animals | | | | Exper. Animals | | | Control Animals | | | |
| | No. 37 | No. 38 | No. 39 | No. 31 | No. 32 | No. 33 | No. 34 | No. 37 | No. 38 | No. 39 | No. 31 | No. 32 | No. 33 | No. 34 |
| Before | 138 | 118 | 130 | 120 | 128 | 132 | 118 | 118 | 90 | 88 | 102 | 110 | 145 | 116 |
| 3 | 160 | 142 | 138 | | | | | | | | | | | |
| 5 | 120 | 110 | 118 | | | 142 | 132 | 140 | 120 | 105 | | | | 122 |
| 10 | 115 | 96 | 118 | 126 | 133 | 140 | 128 | 120 | 112 | 95 | 100 | 109 | 160 | 120 |
| 15 | 110 | 104 | 116 | | | | | 116 | 92 | 95 | | | | |
| 20 | 106 | 108 | 112 | 122 | 130 | 128 | 122 | 116 | 88 | 95 | 102 | 106 | 162 | 122 |
| 25 | 98 | 120 | 110 | | | | | 112 | 96 | 95 | | | | |
| 30 | 96 | 128 | 108 | 120 | 132 | 130 | 120 | 110 | 96 | 95 | 98 | 108 | 160 | 120 |
| 35 | 98 | 130 | 102 | | | | | 112 | 94 | 95 | | | | |
| 40 | 98 | 126 | 100 | 118 | 128 | 130 | 122 | 116 | 96 | 98 | 98 | 108 | 160 | 116 |
| 45 | 100 | 125 | 98 | | | | | 114 | 94 | 96 | | | | |
| 50 | 102 | 125 | 98 | 118 | 128 | 132 | 120 | 116 | 94 | 95 | 102 | 112 | 165 | 118 |
| 55 | 108 | 125 | 104 | | | | | 120 | 94 | 96 | | | | |
| 60 | 110 | 125 | 108 | 120 | 130 | 130 | 122 | 120 | 96 | 98 | 100 | 110 | 165 | 118 |
| 70 | 114 | 125 | 106 | 122 | 132 | 134 | 124 | 128 | 90 | 100 | 104 | 112 | 155 | 116 |
| 80 | 120 | 126 | 108 | 120 | 130 | 140 | 122 | 128 | 92 | 98 | 102 | 110 | 155 | 118 |
| 90 | 118 | 126 | 108 | 124 | 130 | 138 | 122 | 129 | 92 | 96 | 104 | 108 | 155 | 118 |
| 100 | 120 | 126 | 112 | 124 | 132 | 134 | 124 | 128 | 92 | 96 | 104 | 110 | 153 | 120 |
| 110 | 122 | 124 | 110 | 124 | 132 | 138 | 122 | 129 | 94 | 92 | 106 | 110 | 152 | 120 |
| 120 | 118 | 126 | 110 | 124 | 130 | 138 | 122 | 129 | 94 | 96 | 104 | 110 | 150 | 118 |
| Survival Time | 19 hrs. | Survived | 22 hrs. | Survived | Survived | Survived | Survived | 19 hrs. | Survived | 22 hrs. | Survived | Survived | Survived | Survived |

(In cnotrol dogs No. 31 and 32, 5 cc of saline solution alone was infused, and in dogs No. 33 and 34, respectively 5 mg and 25 mg of sodium deoxycholate alone was infused.)

**Tab. 11.** Infusion of Phospholipase A into Portal Vein or Retroperitoneal Space

| | Exper. Dogs | | | Infused | | | Autopsy Findings | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Dog No. | Sex | Body Weight (kg) | Phospholipase A (mg) | Sod. Deoxycholate (mg) | Survival Time (hours) | Liver | Pancreas | Retroperitoneum | Fat Necrosis | Peritoneal Fluid |
| Infusion into Portal Vein | 46 | ♂ | 6.6 | 66 | 25 | 18 | ## | — | — | — | bloody ca. 30cc |
| | 47 | ♀ | 6.3 | 63 | 25 | 21 | ## | — | — | — | bloody ca. 30cc |
| Infusion into Retroperitoneal Space | 37 | ♂ | 7.1 | 5 | 5 | 19 | — | — | Petechia | — | bloody ca. 30cc |
| | 38 | ♀ | 7.2 | 10 | 10 | Survived | — | — | Induration (after 1 week) | — | — |
| | 39 | ♀ | 5.5 | 10 | 10 | 22 | — | — | Petechia | — | bloody ca. 30cc |

(Dog No. 38 was sacrificed 1 week after infusion.)

was infused into the retroperitoneal space and arterial and portal pressures were measured. Little fluctuation was observed except slight increase in arterial pressure after the infusion of sodium deoxycholate, and all the animals survived well.

5. Summary

When phospholipase A was injected intravenously, temporary and marked decrease was observed in arterial pressure in all the animals. On the other hand, different from the intravenous injection into the femoral vein, the injection into the portal vein resulted in death of the animals about 20 hours after the injection, when the influence of marked decrease in arterial pressure seemed to have disappeared. This finding was interpreted that the liver was exposed to the strong influence of phospholipase A directly infused into the portal vein. Autopsy finding revealed an accumulation of approximately 30 cc of bloody peritoneal fluid, and marked change was found in the liver which was dark brown with a tint of light green and with an abnormal glossiness, suggesting intense impairment of the organ. Histologic finding of the liver also revealed edema, congestion and irregular arrangement of hepatic cell cords. Significant change could not be observed in the pancreas, kidney, heart and other.

Such a profound decrease in arterial pressure was not observed, as in the intravenous injection of phospholipase A, when it was injected subcutaneously or into the retroperitoneal space, and in the latter case arterial pressure increased rather reflectively. Portal pressure also slightly increased after the infusion into the retroperitoneal space. These fluctuation within 120 minutes after the infusion, however, can not be accepted to be lethal. Nevertheless, 2 cases out of 3 died about 20 hours after the retroperitoneal infusion of phospholipase A. At autopsy, beside accumulation of 30 cc of bloody peritoneal fluid, petechia could be observed in the retroperitoneal space corresponding to the site of the infusion. However, in the liver, pancreas, kidney, heart and others, marked change could not be observed, without fatty necrosis. Single survivor showed a little lower arterial pressure of

90 mmHg and portal pressure of 70 mm$H_2O$ 1 week after the infusion, and autopsy of this animal revealed only an induration in the site of the infusion of phospholipase A into the retroperitoneal space and peritoneal fluid was hardly observed without marked change in the various organ.

C. Finding of Acute Pancreatitis Produced by Infusion of Phospholipase A

1. Finding of Acute Pancreatitis after Infusion of 0.08 cc/kg Body Weight of Phospholipase A into Pancreatic Duct

0.5 cc (ca. 0.08 cc/kg body weight) of phospholipase A was infused into the main pancreatic duct with 2.5 mg of sodium deoxycholate in anesthetized dog weighing 6.6 kg. The abdomen was opened 24 hours later. Slight edema of the pancreas, its fatty necrosis of the slightest degree and small amount of bloody peritoneal fluid were observed, but hemorrhage or necrosis could not be observed in the pancreas, and the animal survived, as shown in Tab. 12.

2. Finding of Acute Pancreatitis after Infusion of 0.3 cc/kg Body Weight of Phospholipase A into Pancreatic Duct

Into the main pancreatic duct of dogs, 0.3 cc/kg body weight of phospholipase A and about 10 mg (5 mg per 1.0 cc of phospholipase A) of sodium deoxycholate were infused, and the abdomen was opened 24 hours later. In all cases, edema, hemorrhage and fatty necrosis were observed in the pancreas. However, accumulation of bloody peritoneal fluid was slight. Except a single animal with the development of peritonitis, other animals survived, as in Tab. 12.

Instead of sodium deoxycholate, 0.8 cc of autogenous bile was mixed with the equivalent amount of phospholipase A mentioned above was infused into the main pancreatic

**Tab. 12.** Development of Acute Pancreatitis by Infusion of Phospholipase A

| Exper. Dogs | | Infused | | | Pancreatic Changes | | Fat Necrosis | Bloody Peritoneal Fluid | Survival Time (hours) |
|---|---|---|---|---|---|---|---|---|---|
| Dog No. | Body Weight (kg) | Phospholipase A (cc) | Sod. Deoxycholate (mg) | Bile (cc) | Edema | Hemorrhage | | | |
| 64 | 6.6 | 0.5 (0.08cc/kg) | 2.5 | − | + | − | ± | ± | Survived |
| 61 | 6.5 | 2.0 (0.3cc/kg) | 10.0 | − | + | + | + | ± | 90 (Peritonitis) |
| 62 | 6.8 | 2.0 (0.3cc/kg) | 10.0 | − | + | + | + | ± | Survived |
| 65 | 7.5 | 2.3 (0.3cc/kg) | 10.2 | − | + | ± | ± | ± | Survived |
| 63 | 7.2 | 3.6 (0.5cc/kg) | 18.0 | − | + | ++ | + | ± | 30 |
| 66 | 10.2 | 3.1 (0.3cc/kg) | − | 0.8 | ++ | + | + | + | Survived |
| 56 | 6.8 | − | 10.0 (2.0cc) | − | + | − | ± | − | Survived |
| 57 | 6.2 | − | 9.8 (1.9cc) | − | ± | − | − | − | Survived |
| 58 | 8.8 | − | 13.0 (2.6cc) | − | + | − | ± | + | Survived |
| 60 | 10.5 | − | − | 0.8 | + | − | ± | ± | Survived |
| 59 | 9.2 | − | − | 4.6 | ++ | + | + | + | Survived |

duct. The abdomen was opened 24 hours later. Compared with the cases of sodium deoxycholate, marked edema was observed in the pancreas with accumulation of bloody peritoneal fluid, though slight in amount. Hemorrhage and fatty necrosis of such a degree as mentioned above were observed in the pancreas. However, experimental animals all survived, as summarized in Tab. 12.

3. Finding of Acute Pancreatitis after Infusion of 0.5 cc/kg Body Weight of Phospholipase A into Pancreatic Duct

Into the main pancreatic duct of dogs, 0.5 cc/kg body weight of phospholipase A and 18 mg of sodium deoxycholate were infused, and the abdomen was opened 24 hours later. Marked edema and hemorrhage were observed in the pancreas with fatty necrosis. However, the amount of bloody peritoneal fluid was rather small. Experimental animals died 30 hours after the infusion of phospholipase A.

4. Control Study

Sodium deoxycholate alone was dissolved in phosphate buffer so as to be the volume of 0.3 cc/kg body weight and infused into the main pancreatic duct. The abdomen was opened 24 hours later. Slight edema in the pancreas and fatty necrosis of the slightest degree could be observed in some cases, but hemorrhage was not observed, the changes of the pancreas being extremely slight, as shown in Tab. 12. Only in 1 case, however, small amount of bloody peritoneal fluid could be observed.

When autogenous bile of 0.08 cc/kg body weight alone was infused into the main pancreatic duct, laparotomy finding 24 hours after the infusion revealed slight edema of the pancreas, its fatty necrosis of the slightest degree and extremely small amount of bloody peritoneal fluid, but hemorrhage could not be observed, the changes of the pancreas being mild, as shown in Tab. 12.

When autogenous bile of 0.5 cc/kg body weight alone was infused into the main pancreatic duct, marked edema and hemorrhage in the pancreas, its fatty necrosis and accumulation of bloody peritoneal fluid could be observed, 24 hours later, as in Tab. 12. All of the animals which underwent control study survived.

5. Summary

When various amount of phospholipase A was infused into the main pancreatic duct of dogs together with its activator, sodium deoxycholate, changes of the pancreas was to be diverse depending on the dosis of phospholipase A infused. Furthermore, fatty necrosis was commonly observed at the same time, suggesting an important role of phospholipase A in the etiologic process of acute pancreatitis. To compare these finding with those in acute pancreatitis produced by autogenous bile, hemorrhage in the pancreas was more intense, pancreatic edema being milder with less amount of bloody peritoneal fluid, when phospholipase A was infused into the pancreatic duct.

When autogenous bile was mixed instead of sodium deoxycholate, phospholipase A activator, changes were more intense, suggesting a strong activation effect of bile on phospholipase A. Pancreatic edema was particularly outstanding, being accompanied by bloody peritoneal fluid, as in dog No. 66. These changes resembled those of dog No. 59, in which large amount of autogenous bile was infused into the main pancreatic duct. Although it was ascertained that acute pancreatitis can be produced by infusion of phospholipase A and its activator, it was not clarified whether or not the changes be particularly intenser than

when autogenous bile is used, and whether or not acute pancreatitis can be produced with extremely small amount of phospholipas A, in the present experiment. When extremely small amount of phospholipase A was used, changes which deserve the term of acute pancreatitis could not be produced. Hence, it is presumed that phospholipase A does not participate exclusively chemically in the etiologic process of acute pancreatitis, but phospholipase A can produce acute pancreatitis with the aid of mechanical factor, as autogenous bile does.

## IV. DISCUSSION

Principle of pathophysiology of acute pancreatitis can be deemed to consist in the process of activation of pancreatic enzymes and chemical autolysis caused by interstitial emigration of the enzymes. In 1868, KLEBS pointed out the important role of pancreatic enzymes in the etiology of acute pancreatitis for the first time. In the early days of this century, OPIE[68] and OPIE and MEAKINS[69] supported this theory from their studies on regurgitation of bile into the pancreas. Since then, pancreatic enzymes have been variously studied by many researchers from the view-point of its close relationship to acute pancreatitis.

In pancreatic enzymes, amylolytic, lipolytic, proteolytic and nucleolytic enzymes and esterase are contained[97]. These enzymes exist in an inactivated state within the pancreas, and usually activated in the intestine to exert their digestive effect. If these enzymes are activated by some causes within the pancreas and emigrate into the interstitial tissue of the organ, it encounters various disorders such as edema, hemorrhage and necrosis of the pancreas and fatty necrosis in the surrounding tissues. Beside these topical disorders, such general disorders as decrease in arterial pressure and shock state with accumulation of bloody peritoneal fluid were observed, and these constituted characteristic clinical picture of acute pancreatitis. It is generally accepted that amylolytic enzyme manifests little toxicity on organism, and its behavior in acute pancreatitis is extensively investigated. It is widely known that the determination of this enzyme emigrated into blood stream has diagnostic value. Proteolytic enzyme has the most intense toxicity, being the essential factor in pathophysiology of acute pancreatitis. Concerning its behavior in acute pancreatitis, however, opinions do not come to consensus, partly owing to the difficulty of the detection of this enzyme caused by the existence of its inhibitor. Among lipolytic enzymes, serum lipase activity increases at acute pancreatitis and its diagnostic value has been accepted, but in the respect of simplicity of the determination, it has been deemed to be inferior to amylolytic enzyme. However, in 1961, ZIEVE and others[98] postulated diagnostic value of the determination of phospholipase A, from their observation that activity of phospholipase A increases in serum specifically at acute pancreatitis in man.

It has been clarified since early days that the pancreas has the activity to decompose lecithin. In 1877, BÓKAY[10] discovered that pancreatic enzyme decomposes lecithin into fatty acid, choline and glycerophosphate. BELFANTI and NIKUNI[61] independently reported in 1932 that the pancreas has the activity of decomposing lecithin and producing lysolecithin. In 1936, GRONCHI[36] further extracted phospholipase A from pancreatin. During the period from 1950 to 1960, numerous studies have been accumulated in the field of biochemical investigations of phospholipids and phospholipase[16][40][41][55][57][77]. In 1958, RIMON and SHAPIRO[79] separated phospholipase A from bovine pancreas, and in 1961, MAGEE and

others[58] succeeded in purifying phospholipase A from human pancreas. GALLAI-HATCHARD [30] determined in 1965, activity of phospholipase A in various organs of animals, and he observed that the activity was extraordinarily higher in the pancreas than in other organs. Furthermore, VOGEL and ZIEVE[94] observed extremely high activity of phospholipase A in duodenal juice of man, which was followed by the observation of increase in phospholipase A activity in peripheral blood at acute pancreatitis in man, as described in the above.

In the present experiment, activity of phospholipase A was determined following the method of ZIEVE[98]. Similarly to the reports of GALLAI-HATCHARD[30] and ZIEVE[99], the activity was the highest in the pancreatic tissue. It was further observed that this high activity in the pancreas increases to be about 2.5 times after the production of acute pancreatitis. Fluctuation in enzymatic activity within the pancreatic tissue at acute pancreatitis has been variously reported, and the results of the studies on proteolytic enzyme particularly do not coincide with one another. Namely, TURNER and others[92] observed increase in activated trypsin at acute pancreatitis, whereas SUZUKI[87] did not observe trypsin activity, and BECK and others[7][8] reported little difference between the levels of free trypsin, trypsinogen and trypsin inhibitor in extract of the pancreatic tissue at acute biliary pancreatitis and those at normal state.

From the results of the present experiment, it is considered that phospholipase A, activity of which increases within the pancreas, directly emigrates further into portal blood and is distributed to the whole body hematogenously through the liver. Phospholipase A activity in portal blood after production of acute pancreatitis markedly increased and increased on, which approximately coincides with the results of NITTA[62] concerning the behavior of trypsin in pancreatic venous blood. Thus, the liver is exposed to not only trypsin but phospholipase A emigrated from the pancreas.

Increase in phospholipase A activity as observed in peripheral blood in present experiment is considered to be rather lower compared with increas of 10 to 20 times at acute pancreatitis in man as investigated by ZIEVE[98]. However, this might be partly due to the fact that phospholipase A activity is lower in the pancreatic tissue of dogs than in the same tissue of man[99].

It is widely accepted that beside hematogenous route lymphatic route is important as the path-way of general distribution of pancreatic enzymes at acute pancreatitis[35][62][89]. According to ANDERSON and others[3], stream of thoracic duct lymph temporarily increases from 1 to 2 hours after the on-set of acute pancreatitis, but it decreases from 3 to 5 hours after it, thoracic duct lymph becoming bloody, and from 10 to 20 hours later the stream completely stops. Hence, it is presumed that emigration path-way of pancreatic enzymes at acute pancreatitis into portal blood is more important as quantitatively investigated.

In the study of pathophysiology of acute pancreatitis, toxicity of proteolytic enzymes, particularly that of trypsin has been considered to be essential, and the studies have been widely carried out on this respect. Numerous studies have been reported demonstrating important role of trypsin at acute pancreatitis such as decrease in blood pressure[28][73][80][81], alteration in blood coagulation[22][88], thrombus formation[90] and circulatory disturbance due to the contraction of the peripheral vessels[28][54].

On the other hand, REID and others[76] observed that oxygen uptake of homogenate of the kidney, liver and heart was not influenced by the addition of crystalline trypsin,

crystalline chymotrypsin and trypsin inhibitor, which was, however, inhibited by the addition of pancreatic homogenate and vagal juice of the pancreas. ISHIKAWA[50] reported that hepatic tissue respiration of dogs gradually decreased in parallel with the advancement of experimentally produced pancreatic necrosis, but it was not inhibited in normal liver by the addition of trypsin in vitro. From these observations, it is presumed that it might not be trypsin that disturbs the production of cellular oxidative energy, but something else in the pancreas, which influences unfavorably on organism at acute pancreatitis. ISHIKAWA[50], furthermore, clarified that activity of phospholipase C, identical to $\alpha$-toxin of Clostridium Welchii, increases within the liver at acute pancreatic necrosis in dogs. Similarly to this lethal factor, phospholipase A, which acts on phospholipids, also has the toxicity, and it is readily presumed that organism is to be seriously affected when the activity of phospholipase A increases within the body at acute pancreatitis as demonstrated in the present experiment.

Structural integrity of living cells is maintained by the existence of phospholipid[15], and it is easily disturbed, as a matter of course, by the increase in phospholipase A activity with resulting destruction of phospholipid.

From the heat stability of phospholipase A, heated snake venom has been considered to be identical to phospholipase A and widely used in the study of the toxicity. In 1953, BRAGANCA[11] observed that cobra venom treated at 100°C for 15 minutes showed only phospholipase A activity and toxicity in animals, inhibiting oxidation system of glucose, pyruvate, L-glutamate, succinate, $\alpha$-ketoglutarate and fructose in brain homogenate, furthermore strongly inhibiting pyruvic dehydrogenase in the brain, succinic dehydrogenase in the brain and heart muscle, cytochrome oxidase in the brain and choline oxidase in the liver. On the other hand, NYGAARD and SUMNER[64] reported that pancreatic phospholipase A inactivates succinoxidase of liver homogenate of rats and mitochondria of rat liver, as phospholipase A extracted and purified from snake venom does. Moreover, ARAVINDAKSHAN and BRAGANBA[4] reported that heat treated snake venom and crystalline phospholipase have the activity to bring about swelling of mitochondria in mouse liver both in vitro and in vivo and disturbs its metabolism as a result of hydrolysis of phospholipids in the mitochondria. GREIG and GIBBONS[34] observed that hemolysis of human red blood cells treated with lyophilized snake venom was mild, and those red blood cells maintained glycolysis activity, but cholinesterase activity was decreased in those cells with the decrease in the activity to incorporate potassium ion and release sodium ion. In this respect, OZAWA[70] in our clinic, clarified that there occurs disturbance of equilibrium of intracellular ions of sodium and potassium, the cells effluxing potassium ion and influxing sodium ion, with resulting rapid swelling of the cells, when pancreatic mitochondria abundant in phospholipids is affected by phospholipase A.

As has been described in the above, not only phospholipase A itself has strong toxicity, but also when lecithin is decomposed by this enzyme, the product, lysolecithin also has hemolytic activity, as known since early days. HABERMANN[38][39] radically asserted that phospholipase A manifests its pharmacologic effect only by the existence of lecithin. Thus, it is readily presumed that as the activity of phospholipase A increases within the pancreas at acute pancreatitis and emigrates into blood stream to be distributed to the whole body, the liver is firstly exposed to that stream, being seriously affected.

In the present experiment, activity of phospholipase A increased, and there was significant correlation between the degree of the increase in the activity and survival time of experimental animals. The tendency that the earlier and the stronger the increase in the activity was, the earlier occurred the death, was most outstanding in the activity of phospholipase A in portal blood and the liver tissue. In order to ascertain this finding, phospholipase A extracted from bovine pancreas was directly infused into the portal vein. In this experiment, temporary decrease in blood pressure was observed as in the experiment of intravenous infusion of phospholipase A into the femoral vein. However, experimental animals exclusively with phospholipase A infusion into the portal vein died approximately 20 hours after the infusion, revealing autopsy finding of marked changes in the liver. CONDREA[12] and others reported that they observed decrease in plasma lecithin and transient increase in lysolecithin with deformity of red blood cells in their experiment in which 50 mg (protein equivalent) of phospholipase A extracted from bovine pancreas was intravenously injected into the auricular vein in rabbits for 2 times with the interval of time of 45 minutes, however, without death of the animals. As compared with the dosis of phospholipase A in the experiment of CONDREA, less than one half of phospholipase A in proportion to body weight was injected into the femoral vein without death of the experimental animals in the present experiment. However, the same amount of the enzyme was lethal when infused into the portal vein. From this finding, it is obviously suggested that the liver is seriously affected by this enzyme. OHARA[67] observed blood congestion in the central vein and sinusoid and atrophy of hepatic cell cord 8 hours after production of acute pancreatitis, and he further reported that these findings could be observed for 1 to 3 days which was followed by improvement of blood congestion and atrophic picture in parallel with the improvement of general condition, and the changes in the liver were more serious in dogs died within 5 days after the production of acute pancreatitis than those slaughtered on purpose. On the other hand, ANDERSON[2] experimentally produced various degree of acute pancreatitis from slight edema to serious necrosis of the pancreas and investigated morphologic and functional changes of the liver. According to his observation, histologic findings of the liver such as isolation of hepatic cell cords, blood congestion in the sinusoids and degeneration or necrosis of hepatic cells were in parallel with the degree of the pancreatic lesion, and liver fuction, as in vestigated by alkaline phosphatase, T. T. T., s-G. O. T. and s-G. P. T., was similarly disturbed depending on the degree of pancreatic changes.

Pathogenesis of hepatic disturbance at acute pancreatitis has been much discussed, and it is presumed that in addition to circulatory disturbance within the liver associated with decrease in general blood pressure, trypsin emigrated from the pancreas to the pancreatic vein reaches the liver via the portal vein[49)62)74)], with resulting circulatory disturbance of the liver[54)]. Thus, the liver is placed in a condition of hypoxia, and proteolytic effect of trypsin, influence of phospholipase A on membranous components of cells, particularly disturbance of mitochondria and further disturbance of energy metabolism[4)11)64)] might come to play the important role in etiologic pathophysiology of acute pancreatitis.

Furthermore, DE DUVE[17] demonstrated that acid hydrolase such as cathepsin is contained in lysosomes of the liver, and they clarified that cellular autolysis advances by the activation of catheptic system, caused by the release of these enzymes[18]. VAN LANCKER and HOLTZER[93] demonstrated the existence of acid hydrolases, which were probably

lysosomal enzymes, in the pancreatic tissue. The membrane of this lysosome also contains lipoprotein-barrier, which is destructed by phospholipase with resulting release of lysosomal enzymes and, further, activation of these enzymes. Such process is also presumed to be a factor of the disturbance. BERRIDGE and WATMAN[9] and RYAN[84] pointed out that at acute pancreatitis increased fragility of red blood cell would develop to hemolytic jaundice, and they interpreted this phenomenon to be due to influence of trypsin on these cells. However, this can be differently interpreted that increase in the activity of phospholipase A in blood at acute pancreatitis might be indirect cause of the jaundice, since lysolecithin with its strong effect of hemolysis is produced from lecithin by phospholipase A.

It is generally known that large amount of bloody exudative fluid accumulates in the peritoneal cavity and retroperitoneal space by the increase in capillary permeability, increase in portal pressure and obstruction of the lymphatics, with resulting decrease in circulating plasma volume, which constitutes a factor of shock development. In this exudative fluid, increase in activity of amylase and lipase has been pointed out. In the present experiment also, activity of phospholipase A was markedly higher in peritoneal fluid than in serum, further increasing on in parallel with the development of changes of the pancreas. Existence of vasoactive substance and abnormal albumin was demonstrated by NUGENT[63], KATZ and others[52], and RODGERS and CAREY[82] in peritoneal fluid. TSUKIYAMA[91] also asserted that toxic substances exist in peritoneal fluid and the toxicity consists in the mixture of the three of trypsin, amylase and lipase. In the present experiment, such a clear correlation could not be observed between the degree of increase in phospholipase A activity in peritoneal fluid, as was observed between the degree of increase in the activity in portal blood or in the hepatic tissue and the survival time. However, it is considered that the toxicity of increased phospholipase A activity in peritoneal fluid should not be estimated too small.

It is generally observed that a burn-like injury occurs in the retroperitoneal space at acute pancreatitis, and in this respect the importance of fluid transfusion has been pointed out[32]. Thus, infiltration of exudative fluid into the retroperitoneal space has the important significance in pathophysiology of acute pancreatitis. NAKAGAWA[59] sutured and fixed the pancreas of dogs to the retroperitoneum to simulate the anatomical situation of human pancreas, and he succeeded in producing extremely serious shock state by infusion of such small amount of bile into the main pancreatic duct, as commonly leading to a fail to produce serious acute pancreatitis. He further ascertained that shock state could be produced by infiltration of trypsin around the abdominal aorta. In the present experiment also, phospholipase A was injected and infiltrated around the abdominal aorta. However, such a rapid fluctuation in arterial and portal pressures could not be observed as was observed when trypsin was used. Despite the shock state could not be observed in these animals for 2 hours' investigation, 2 animals out of 3 died about 20 hours after the infusion of phospholipase A into the retroperitoneal space. Cause of death can not be explained only by this finding, but the importance of direct effect of phospholipase A on the nervous plexus around the abdominal aorta should be emphasized, considering the fact that the same amount of phospholipase A showed little effect in dogs when injected subcutaneously, together with the fact that subcutaneous injection of heat treated snake venom results in swelling of mitochondria in the brain and liver, disturbing the function of these organs[4][11].

GILSDORF[31] and OGAWA[66] succeeded in producing and developing acute pancreatitis by electric stimulation to the hypothalamic area, splanchnic nerves or celiac nervous plexus. From these results, they emphasized neurogenic factor in pathophysiology of acute pancreatitis.

One of the characteristic findings of acute pancreatitis is fatty necrosis. This has been considered to occur by the principal effect of lipase, but according to the experiment of PANABOKKÉ[71], it was difficult to produce fatty necrosis by injection of lipase or trypsin into the adipose tissue, whereas injection of phospholipase into this tissue could produce it. Hence, it is assumed that lipase can manifest its activity only when phospholipase A previously affected the cellular membrane of the adipose tissue, and participation of phospholipase in the development of fatty necrosis should not be neglected.

In 1901, OPIE[68] asserted "common channel theory", in which the etiologic pathophysiology of serious acute pancreatitis is assumed to consist in regurgitation of bile into the pancreatic duct through the common canal formed with the bile duct and pancreatic duct by the incarceration of gall stone in the ampulla of VATER. In 1919, ARCHIBALD[5] reported that the common channel can be formed functionally not only by incarceration of gall stone, but also by inflammation or hyperactivity of the duodenum, stimulation from the biliary system, dysfunction of autonomic nerves, mental irritation, spasm of ODDI's muscle caused by drugs such as morphine and edema of papilla of VATER. These assertions were followed by many studies thereafter concerning the relationship between acute pancreatitis and regurgitation of bile into the pancreatic duct. As pancreatic enzymes participating in pathophysiology of acute pancreatitis, trypsin has been and is considered to be essential[53][69][75][78][85]. However, there are some contradictions upon this concept, and questions on the effect of trypsin, particularly at the initial stage of the etiologic process and its association with bile are not yet clarified. DRAGSTEDT[20] pointed out, in 1934, that trypsin does not act on lipoids, and he isolated small part of the pancreas of dog preserving blood supply and transferred it into the duodenal canal to expose the pancreatic tissue to trypsin. The experiment was, however, unsuccessful to produce acute pancreatitis, whereas similarly transferred pancreatic tissue into the gall bladder was affected. SUZUKI[87] observed that trypsin cannot be detected in the pancreatic tissue with biliary acute pancreatitis, and changes in the pancreas was milder when emulsion of intestinal membrane with stronger activation effect on trypsinogen was infused into the pancreatic duct than when bile was used, which has lesser effect of activation of trypsinogen. Furthermore, FUJIYAMA[29] asserted that increase in trypsin cannot be demonstrated in pancreatic juice of dogs with acute pancreatic necrosis, though with increase in amylase and lipase, and he pointed out that injection of enterokinase solution into the pancreatic parenchyma failed to produce pancreatic necrosis, although an increase in trypsin was observed. Both of these researchers are skeptic to assume the role of trypsin to be essential. HAVERBACK and others[43][44] pointed out that trypsinogen is not activated by bile and insisted the existence of non-trypsin pancreatitis basing on their investigations on trypisn inhibitor. BECK[7][8] also insisted that even administration or direct infusion of extremely large amount of trypsin inhibitor into the pancreatic duct such as benthonium chloride or trasylol cannot prevent the occurrence of experimental acute pancreatitis in rabbits and dogs, and they observed little difference between the levels of free trypsin, trypsinogen and trypsin inhibitor in pancreatic extract from the animals with acute biliary pancreatitis and those from normal dogs. From the morphologic investigation of

various organs in experimental acute biliary pancreatitis in dogs, WANKE and GROEZINGER[95] pointed out the difference between biliary pancreatitis and trypsin pancreatitis. ELMSLIE [25)26)] maintained that acute pancreatitis does not develop even by infusion of activated trypsin into the pancreatic duct, unless it is not infused with pressure.

NEMIR and DRABKIN[60] put emphasis on hemochromogen from their observation that fulminant pancreatitis could be produced by the infusion of blood digested with pancreatic juice into the pancreatic duct, and they considered that hemochromogen plays an important role not only as an etiologic factor of acute pancreatitis, but in its pathophysiology. HOFERICHTER[46] also noticed the importance of digested blood. ELLIOTT[24] reported the occurrence of fulminant pancreatitis by the use of a mixture of the same amount of bile and pancreatic juice incubated for 12 to 48 hours. On the other hand, CREUTZFELDT[13] reported that he failed to produce serious acute pancreatitis by the infusion of activated trypsin, mixture of activated trypsin and blood, lipase and other various materials into the pancreatic duct, and fulminant acute pancreatitis could be produced only when lysolecithin was infused into the pancreatic duct. He further pointed out that most part of lecithin commonly demonstrated in normal pancreatic tissue is transformed to lysolecithin in pancreatic tissue with experimental and clinical acute pancreatitis, and he succeeded in producing similarly fulminant acute pancreatitis by the infusion of phospholipase A, which participates in the production of lysolecithin, with cholic acid into the pancreatic duct. Furthermore, he postulated that the significance of phospholipase A is extremely important in etiologic process of acute pancreatitis, since phospholipase A is far abundantly contained in human pancreas than in animal's one, fulminant acute pancreatitis is frequently observed in man, which is relatively rare in animals, and activity of phospholipase A is elevated by the regurgitation of bile and/or duodenal juice into the pancreas.

In the present experiment, phospholipase A extracted from bovine pancreas was infused into the pancreatic duct in various dosis with sodium deoxycholate, and various degree of acute pancreatitis could be produced depending on the dosis of phospholipase A. However, the amount of simultaneously infused sodium deoxycholate was obviously smaller than the amount which alone can produce acute pancreatitis[45], and it is readily presumed that phospholipase A is an important factor in the occurrence of acute pancreatitis.

On the other side, GREENBAUM and HIRSHKOWITZ[33] demonstrated that trypsinogen is activated in vitro by pancreatic cathepsin, and TURNER[92] postulated that as the catheptic system consisted from lysosomal enzymes is activated by the decrease in intracellular pH caused by hypoxic conditions or ischemia, trypsinogen is activated in vivo also along with autolysis inducing necessarily resulting occurrence and development of acute pancreatitis. As has been described in the above, lysosom membrane has lipoprotein-barrier, and in this respect it is assumed that phospholipase A presumably participates in this process.

Several unexplored problems in the explanation of etiology of acute pancreatitis, as has been done attributing the essential factor to trypsin and regurgitation of bile into the pancreatic duct, can be clarified, to some extent, by introducing the concept of participation of phospholipase A. By the regurgitation of bile abundant in bile acid, phospholipase A in pancreatic juice is activated, which then directly affect membranous components of cells, or lecithin, abundantly contained in pancreatic tissue or bile, is decomposed and lysolecithin is produced, which indirectly acts on the cells. It is assumed that the cells are

thus destructed and ruptures occur in the small branches of the pancreatic duct. Thus, once injured cells are further affected by proteolytic enzyme, and trypsinogen is activated by lysosomal enzymes, along with activation of trypsinogen by trypsin itself[44].

From experimental results mentioned above, it is assumed that pathophysiologic process advances centering around the principal role of trypsin, under previously induced pathologic condition triggered by phospholipase A, with resulting further development of acute pancreatitis.

## V. SUMMARY

It has been considered that various pancreatic enzymes are activated at acute pancreatitis and emigrates from the pancreas, being distributed to the whole body to present characteristic clinical picture of the disease, and these enzymes have important influence on the prognosis. Phospholipase A has been said to be abundantly contained in the pancreas and studied on its toxicity, but this enzyme has not been fully evaluated from the view-point of etiologic factor and pathophysiologic role in acute pancreatitis. In the present experiment, acute biliary pancreatitis was produced in dogs and behavior of phospholipase A was studied in these animals. Further investigations were carried out on the direct influence of phospholipase A on organism, and obtained results are summarized as follows:

1) Activity of phospholipase A was extremely high even in the pancreas of normal dogs. This activity in the pancreas increased as acute pancreatitis was produced, with the simultaneous increase in the activity of phospholipase A in the liver, portal blood, peripheral blood and peritoneal fluid, the degree of increase being most outstanding in portal blood.

2) There was a tendency that the earlier and the stronger was the increase in the activity of phospholipase A, the earlier the animals died. This finding was most remarkable as to the activity of phospholipase A in portal blood and the liver.

3) When phospholipase A was infused into the portal vein or femoral vein of dogs, marked decreased in arterial pressure was temporarily observed, which shortly restored to normal level without falling into shock state. Experimental animals with phospholipase A infusion into the femoral vein improved thereafter and survived, whereas those with the infusion into the portal vein died approximately 20 hours later. Autopsy findings of these animals revealed marked changes in the liver.

4) Phospholipase A infusion into the retroperitoneal space resulted in little fluctuation of blood pressure for 2 hours after the infusion. Two experimental animals out of 3, however, died approximately 20 hours after the infusion. Autopsy finding of these 2 animals revealed little change in the pancreas, liver and other organs.

5) Various amount of phospholipase A was infused into the pancreatic duct of dogs together with sodium deoxycholate. Acute pancreatitis of various degree could be observed depending upon the amount of infused phospholipase A.

From these findings, it is assumed that as phospholipase A in the pancreas with its increased activity at acute pancreatitis emigrates into portal blood to reach the liver, or exudative fluid with high activity of phospholipase A infiltrates directly in the retroperitoneal space, organism receives serious influence. It is also assumed that phospholipase A plays an important role in the etiologic process of acute pancreatitis.

As was clarified in the present experiment, phospholipase A plays never less im-

portant role than trypsin, even though not more, in etiologic process and pathophysiology of acute pancreatitis, and it is assumed that several unexplored problems in the explanation of etiology and pathophysiology of acute pancreatitis, as has been done attributing the principal factor to trypsin, can be explained to some extent by introducing the concept of pariticipation of phospholipase A.

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## VI. REFERENCES

*1) Akabori, S. : Kooso-kenkyuho II. III. Asakura-shoten, Tokyo, 1956.
2) Anderson, M. C. : Hepatic morphology and function. Alterations associated with acute pancreatitis. Arch. Sur., **92** : 664, 1966.
3) Anderson, M. C., Schoenfeld, F. B., Iams, W. B. and Suwa, M. : Circulatory changes in acute pancreatitis. Sur. Clin. N. Amer., **47** : 127, 1967.
4) Aravindakshan, I. and Braganca, B. M. : Studies on phospholipid structures in mitochondria of animals injected with cobra venom or phospholipase A. Biochem. J., **79** : 84, 1961.
5) Archibald, E. W. : The experimental production of pancreatitis in animals as the result of the resistance of the common duct sphincter. Surg. Gynec. & Obst., **28** : 529, 1919.
6) Beaufay, H. and de Duve, C. : Tissue fractionation studies. 9. Enzymic release of bound hydrolases. Biochem. J., **73** : 604, 1959.
7) Beck, I. T., Pinter, E. J., Solymar, J., McKenna, R. D. and Ritchie, A. C. : The role of pancreatic enzymes in the pathogenesis of acute pancreatitis. II. The fate of pancreatic proteolytic enzymes in the course of acute pancreatitis. Gastroenterology, **43** : 60, 1962.
8) Beck, I. T., Pinter, E., McKenna, R. D,. Ritchie, A. C. and Solymar, J. : The fate of pancreatic proteolytic enzymes in the course of acute hemorrhagic pancreatitis. The 2nd World Congress of Gastroenterology, Munich, 1962. IV. Topical problems in pancreatitis. : 113, S. Kager, Basel, New York, 1963.
9) Berridge, F. E. J. and Watman, R. N. : Hemolytic aspects of acute pancreatitis. Surg. Forum, 43rd. Clinical Congress, **7** : 255, 1957.
10) Bōkay, A. : Ueber die Verdaulichkeit des Nucleins und Lecithins. Zschr. Physiol. Chem., **1** : 157, 1877.
11) Braganca, B. M. and Quastel, J. H. : Enzyme inhibitions by snake venoms. Biochem. J., **53** : 88, 1953.
12) Condrea, E., Klibansky, C,. Keret, R. and de Vries, A. : Action of bovine and human pancreatic phospholipase A on lipoprotein-bound phospholipids. Nature, **200** : 1096, 1963.
13) Creutzfeldt, W. : The pathogenesis of pancreatitis. The 3rd World Congress of Gastroenterology, Tokyo, 1966.
14) Creutzfeldt, W., Soeling, H. D. und Ketterer, H. : Intrapankreatische Aktivierung von proteolytischen Fermenten und Trasylol-wirkung bei der experimentellen Pankreatitis der Ratte. Klin. Wschr., **41** : 1002, 1963.
15) Dawson, R. M. C. : The animal phospholipase. Their structure, metabolism and biological signifigance. Biol. Rev., **32** : 188, 1957.
16) Dawson, R. M. C. : On the mechanism of action of phospholipase A. Biochem. J., **88** : 414, 1963.
17) de Duve C., Pressman, B. C., Gianetto, R., Wattiaux, R. and Appelmans, F. : Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat-liver tissue. Biochem. J,. **60** : 604, 1955.
18) de Duve, C. and Beaufay, H. : Tissue fractionation studies. 10. Influence of ischemia on the state of some bound enzymes in rat-liver. Biochem. J., **73** : 610, 1959.
19) Dragstedt, L. R. : The digestion of living tissues by proteolytic enzymes. Science, **98** : 131, 1943.
20) Dragstedt, L. R., Haymond, H. E. and Ellis, J. C. : Pathogenesis of acute pancreatitis (acute pancreatic necrosis). Arch. Surg., **28** : 232, 1934.
21) Dreiling, D. A., Janowitz, H. D. and Perrier, C. V. : Pancreatic inflammatory disease. Hoeber, Harper & Row. New York, 1964.

22) Eagle, H. and Harris, S. T. : Studies in blood coagulation. I. The coagulation by proteolytic enzyme. J. Gen. Physiol., **20** : 543, 1937.
23) Edwards, S. W. and Ball, E. G. : The action of phospholipases on succinate oxidase and cytochrome oxidase. J. Biol. Chem., **209** : 619, 1954.
24) Elliott, D. W., Williams, R. D. and Zollinger, R. M. : Alterations in the pancreatic resistance to bile in the pathogenesis of acute pancreatitis. Ann. Surg., **146** : 669, 1957.
25) Elmslie, R. and White, T. T. : An Investigation into trypsin in the pancreaticobiliary tree. Brit. J. Surg., **53** : 586, 1966.
26) Elmslie, R., White, T. T. and Magee, D. F. : The significance of reflux of trypsin and bile in the pathogenesis of human pancreatitis. Brit. J. Surg., **53** : 809, 1966.
27) Fiske, C. H. and Subbarow, Y. : The colorimetric determination of phosphorus. J. Biol. Chem., **66** : 375, 1925.
28) Forell, M. M. : Zur Frage des Entstehungsmechanismus des Kreislaufkollapses bei der akuten Pankreasnekrose. Gastroenterologia, **84** : 225, 1955.
*29) Fujiyama, S. : Experimental studies on the acute pancreatic necrosis. Okayama,Igakukai-Zasshi, **53** : 1848, 1941.
30) Gallai-Hatchard, J. J. and Thompson, R. H. S. : Phospholipase A activity of mammalian tissues. Biochem. Biophys. Acta, **98** : 128, 1965.
31) Gilsdorf, R. B., Long, D., Moberg, A. and Leonard, A. S. : Central-nervous-system influence on experimentally induced pancreatitis. J. A.. M. A., **192** : 394, 1965.
32) Gray, S. H. and Rosenman, L. D. : Acute pancreatitis. The significance of hemoconcentration at admission to the hospital. Arch. Surg., **91** : 485, 1965.
33) Greenbaum, L. M. and Hirshkowitz, A. : Endogenous cathepsin activation of trypsinogen in extracts of dog pancreas. Proc. Soc. Exper. Biol. & Med., **107**, 74, 1961.
34) Greig, M. E. and Gibbons, A. J. : Cation transport in erythrocytes treated with lecithinase A. Arch. Biochem. Biophys., **61** : 343, 1956.
35) Grenier, J. F., Gillet, M., Hatano, M. and Weise, A. G. : La toxémie dans les pancréatities aigues. Etude clinique et expérimentale. The 3rd World Congress of Gastroenterology, Tokyo, 1966.
36) Gronchi, V. : Ricerche sulla lecitinasi ≪A≫ del pancreas. Sperimentale, **90** : 223, 1936.
37) Guleke, N. : Über Die experimentelle Pankreasnekrose und die Todesursache bei akuten Pankreas-erkrankungen. II. Theil. Arch. Klin. Chir., **85** : 615, 1908.
38) Habermann, E. : Beiträge zur Pharmakologie von Phospholipase A. Arch. exper. Path. Pharmakol., **230** : 538, 1957.
39) Habermann, E. und Krusche, B. : Wirkung der Phospho lipasen A und C auf Plasmalipoide und Erythrozyten in vivo. Biochem. Pharmakol., **11** : 400, 1962.
40) Hanahan, D. J. : The enzymatic degradation of phosphatidyl choline in diethyl ether. J. Biol. Chem., **195** : 199, 1952.
41) Hanahan, D. J., Rodbell, M. and Turner, L. D. : Enzymatic formation of monopalmitoleyl- and monopalmitoyl lecithin (lysolecithins). J. Biol. Chem., **206** : 431, 1954.
42) Hansson, K., Lundh, G. and Stenram, U. : Morphological and secretory studies in experimentally induced pancreatitis in rats. Acta Chir. Scand., **121** : 274, 1961.
43) Haverback, B. J. : Exocrine function of the pancreas. Reappraisal of some physiological and biochemical principles. J. A. M. A., **193** : 279, 1965.
44) Haverback, B. J., Dyce, B., Bundy, H. and Edmondson, H. A. : Trypsin, trypsinogen and trypsin inhibitor in human pancreatic juice. Amer. J. Med., **29** : 424, 1960.
*45) Hayano, Z. : Studies on experimental pancreatitis. Hokkaido-Igaku, **36** : 89, 1961.
46) Hoferichter, J. : Klinische und experimentelle Unter suchunged zur akuten haemorrhagischen Pankreasnekrose. Langenbecks Arch. Klin. Chir., **307** : 319, 1964.
*47) Honjo, I. : Symposium on pancreatitis. The 16th Gen. Ass. Jap. Med. Cong., 1963.
48) Howard, J. M. and Jordan, G. L. : Surgical diseases of the pancreas. Lippincott, Philadelphia, 1960.
49) Howard, J. M., Smith, A. K. and Peters, J. J. : Acute pancreatitis : pathway of enzymes into the blood stream. Surgery, **26** : 161, 1949.
50) Ishikawa, T. : Pathophysiology of the liver and pancreas in experimentally produced acute pancreatic necrosis and therapeutic effect of penicillin. Arch. Jap. Chir., **34** : 287, 1965.

51) Janowitz, H. D. and Hollander, F. : The exocrine-endocrine partition of enzymes in the digestive tract. Gastroenterology, **17** : 591, 1951.
52) Katz, N., Silverstein, M., Kobold, E. E. and Thal, A. P. : Trypsin release, kinin production and shock. Relationship in experimental and human pancreatitis. Arch. Surg., **89** : 322, 1964.
53) Keith, L. M. J., Barnes, J. E. and Denkewalter, F. R. : Experimental study of interstitial injection of trypsin and trypsinogen into the pancreas. A. M. A. Arch. Surg., **77** : 416, 1958.
54) Kinami, Y. : An experimental study on the influence of trypsin on peripheral circulation. Arch. Jap. Chir., **34** : 257, 1965.
55) Long, C. and Penny, I. F. : The structure of the naturally occuring phosphoglycerides. 3. Action of moccasin-venom phopholipase A on ovolecithin and related substances. Biochem. J., **65** : 382, 1957.
56) Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. : Protein measurement with the folin phenol reagent. J. Biol. Chem., **193** : 265, 1951.
57) Magee, W. L. and Thompson, R. H. S. : The estimation of phospholipase A activity in aqueous systems. Biochem. J., **77** : 526, 1960.
58) Magee, W. L., Gallai-Hatchard, J., Sanders, H. and Thompson, R. H. S. : The purification and properties of phospholipase A from human pancreas. Biochem. J., **83** : 17, 1962.
59) Nakagawa, T. : Experimental studies on the significance of pathologic change in retroperitoneum at acute pancreatitis. Arch. Jap. Chir., **35** : 659, 1966.
60) Nemir, P. J. and Drabkin, D. L. : The pathogenesis of acute necrotizing hemorrhagic pancreatitis. An experimental study. Surgery, **40** : 171, 1956.
61) Nikuni, Z. : On the formation of lysolecithin from eggyolk lecithin by pancreas extract. Bull. Agr. Chem. Soc. Jap., **8** : 104, 1932.
62) Nitta, T. : Attitude of trypsin in experimental acute pancreatitis. Arch. Jap. Chir., **33** : 703, 1964.
63) Nugent, F. W. : The 3rd World Congress of Gastroenterology, Scientific exhibition. Tokyo, 1966.
64) Nygaard, A. P. and Sumner, J. B. : The effect of lecithinase A on the succinoxidase system. J. Biol. Chem., **200** : 723, 1953.
65) Obata, K. : Attitude of trypsin inhibitor at acute pancreatitis. Arch. Jap. Chir., **34** : 55, 1965.
66) Ogawa, Y., Hori, M., Yamazaki, Z., Fujimori, Y. and Hatano, S. : Role of pancreatic blood flow and pancreatic nerve in acute pancreatitis. The 3rd World Congress of Gastroenterology, Tokyo, 1966.
*67) Ohara, T. : Studies on experimental acute pancreatitis. Yamaguchi-Igaku, **13** : 183, 1964.
68) Opie, E. L. : Relation of cholelithiasis to disease of the pancreas and to fat necrosis. Amer. J. Med. Sci., **121** : 27, 1901.
69) Opie, E. L. and Meakins, J. C. : Data concerning the etiology and pathology of hemorrhagic necrosis of the pancreas. J. Exp. Med., **11** : 561, 1909.
*70) Ozawa, K. : Personal communication.
71) Panabokké, R. G. : An experimental study of fat necrosis. J. Path. Bact., **75** : 319, 1958.
72) Pangborn, M. C. : A simplified purification of lecithin. J. Biol. Chem., **188** : 471, 1951.
73) Peck, M. E. : Preliminary observations on the effect of trypsin administered intravenously. J. A. M. A., **154** : 15, 1954.
74) Popper, H. L. and Necheles, H. : Pathways of enzymes into the blood in acute damage of the pancreas. Proc. Soc. Exp. Biol. & Med., **43** : 220, 1940.
75) Powers, S. R. J., Browne, H. H. and Stein, A. A. : The pathogenesis of acute and chronic pancreatitis. Ann. Surg., **142** : 690, 1955.
76) Reid, L. C., Paulette, R. E., Challis, T. W. and Hinton, J. W. : The mechanism of the pathogenesis of pancreatic necrosis and the therapeutic effect of propylthiouracil. Surgery, **43** : 538, 1958.
77) Rhodes, D. N. and Lea, C. H. : Phospholipids. 4. On the composition of hen's egg phospholipids. Biochem. J., **65** : 526, 1957.
78) Rich, A. R. and Duff, G. L. : Experimental and pathological studies on the pathogenesis of acute hemorrhagic pancreatitis. Bull. Johns Hopkins Hosp., **58** : 212, 1936.
79) Rimon, A. and Shapiro, B. : Properties and specificity of pancreatic phospholipase A. Biochem. J., **71** : 630, 1959.
80) Rocha e Silva, M. : Beiträge zur Pharmakologie des Trypsins. II. Wirkung des Trypsins auf den Blutkreislauf bei Katze, Kaninchen und Hund. Arch. Exp. Path. Pharmakol., **194** : 351, 1940.
81) Rocha e Silva, M., Beraldo, W. T. and Rosenfeld, G. : Bradykinin, a hypotensive and smooth muscle

stimulating factor released from plasma globulin by snake venoms and by trypsin. Amer. J. Physiol., **156** : 261, 1949.

82) Rodgers, R. E. and Carey, L. C. : Peritoneal lavage in experimental pancreatitis in dogs. Amer. J. Surg., **111** : 792, 1966.

83) Rush, B. J. and Clifton, E.F. : The role of trypsin in the pathogenesis of acute hemorrhagic pancreatitis and the effect of an anti-tryptic agent in treatment. Surgery, **31** : 349, 1952.

84) Ryan, J. W., Bethesda, Moffat, J. C. and Thompson, A. G. : Role of bradykinin system in acute hemorrhagic pancreatitis. Arch. Surg., **91** : 14, 1965.

85) Stein, A. A., Powers, S. R. J. and Browne, H. H. : Experimental hemorrhagic pancreatitis : new concepts of pathogenesis. Ann. Surg., **143** : 508, 1956.

86) Stern, L. and Shapiro, B. : A rapid and simple method from the determination of esterified fatty acid and total fatty acid in blood. J. Clin. Path., **6** : 158, 1953.

*87) Suzuki, T. : Experimental studies on the pathogenesis of acute pancreatic necrosis and on the pancreatic toxin. Okayama-Igakukai-Zasshi, **52** : 1217, 1940.

88) Tagnon, H. J. : The nature of the mechanism of the shock produced by the injection of trypsin and thrombin. J. Clin. Invest., **24** : 1, 1945.

*89) Takizawa, K. : Experimental studies on the appearing route of pancreatic diastase into blood stream. Tohoku-Igaku, **57** : 179, 1958.

90) Taylor, A. and Wright, I. S. : Intravenous trypsin. Circulation, **10** : 331, 1954.

91) Tsukiyama, Y. : Disturbance of the liver, kidney and heart in cases of acute pancreatitis. The 3rd World Congress of Gastroenterology, Tokyo, 1966.

92) Turner, M. D., Correll, W. W., Fain, W. R., Conn, J. H. and Cockrell, J. V. : Enzyme levels in pancreatic tissue and body fluids during experimental pancreatitis. J. Sur. Res.. **3** : 485, 1963.

93) Van Lancker, J. L. and Holtzer, R. L. : Tissue fractionation studies of mouse pancreas. Intracellular distribution of nitrogen, deoxyribonucleic acid, ribonucleic acid, amylase, acid phosphatase, deoxyribonuclease and cytochrome oxidase. J. Biol. Chem., **234** : 2359, 1959.

94) Vogel, W. C. and Zieve, L. : Lecithinase A in duodenal contents of man. J. Clin. Invest., **39** : 1295, 1960.

95) Wanke, M. und Groezinger, K. H. : Organveränderungen bei experimenteller Pankreatitis. Mit besonderer Berücksichtigung der Leber-Pankreasbeziehung. Langenbecks Arch. Klin. Chir., **310** : 36, 1965.

96) Yamabe, I. : Study on lecithinase C activity in the liver necrosis after interruption of the arterial flow to the liver. Arch. Jap. Chir., **29** : 205, 1960.

97) Zieve, L. : Pancreatic exzymes : clinical value of determinations of various pancreatic enzymes in serum. Gastroenterology, **46** : 62, 1964.

98) Zieve, L. and Vogel, W. C. : Measurement of lecithinase A in serum and other body fluids. J. Lab. Clin. Med., **57** : 586, 1961.

99) Zieve, L., Vogel, W. C. and Kelly, W. D. : Species difference in pancreatic lipolytic and amylolytic enzymes. J. Appl. Physiol., **18** : 77, 1963.

(*in Japanese)

和文抄録

# 急性膵炎の病因及び病態生理

## 殊に phospholipase A の関与について

京都大学医学部外科　第1講座(指導：本庄一夫教授)

畑　尾　正　彦

従来より，急性膵炎の病因あるいは病態生理が論ぜられる場合，蛋白分解酵素殊に trypsin が演ずる役割の極めて重要なことは，一般に広く認められているところである．一方，Dragstedt は trypsin が lipoids には作用せず，また細胞膜が lipoid material により構成されていることから，この trypsin を中心とせる概念にはじめて疑問をいだいたが，その後，鈴木，藤山，Haverback, Beck らは胆汁性膵炎における膵組織中または膵液中に trypsin 活性を認めず，更に形態学的にも胆汁性膵炎は trypsin によるそれとは異なつていることが指摘され，また活性 trypsin の膵管内活入が必ずしも膵炎を惹起しないなど，trypsin一辺倒の考え方に対する批判も少なくない．

石川は，犬の実験的急性膵壊死において，肝内に phospholipase C 活性が亢進して致死的因子となり得ることを示したが，膵に，この lethal factor と等しく phospholipid に作用する phospholipase A の豊富に存在することが Bókay の報告以来，広く知られて居り，また phospholipase A に強い毒性のあることか，Braganca, Aravindakshan, Nygaard and Sumner, Greig and Gibbons らによつて明らかにされている．

急性膵炎の際，各種の膵酵素が活性化され間質内に逸脱して化学的自己融解現象を招き，あるいは全身に拡がつて，この疾病に特有の病像を形づくり，予後に重大な影響を及ぼすものと考えられているが，膵に本来，豊富に存在し，しかも強い毒性を有するものであるといわれながら phospholipase A に関しては，これまで，あまりかえりみられていなかつた．今回の実験では，犬に胆汁性の急性膵炎を作成して，phospholipase A 活性の動態を検索し，更にこの酵素の直接生体に及ぼす影響についても検討を加え，次の結果を得た．

1)　正常の犬でも，膵に極めて高い phospholipase A 活性が認められた．急性膵炎を作成すると，この膵内 phospholipase A 活性は更に亢進したか．それと同時に，肝，門脈血，末梢血，腹腔液中の phospholipase A 活性も亢進し，その亢進の程度は，門脈血において最も著明であつた．

2)　早期に強く phospholipase A 活性の亢進せるものほど，膵炎作成後．短時間に死亡するという傾向が認められ，これは門脈血及び肝において最も明瞭であつた．

3)　phospholipase Aを犬の門脈内または大腿静脈内に注入すると一過性に著明の動脈圧下降が認められたが，これは間もなく正常域に復しそのまま shock に陥ることはなかつた．大腿静脈内注入犬は，その後元気に回復，生存したか，門脈内注入犬は注入後約20時間で死亡した．剖検で肝に強い変化が認められた．

4)　phospholipase A を犬の後腹膜に注入したところ，注入後2時間の観察では殆んど血圧の変動を示さなかつたが，約20時間後には，3頭の実験犬のうち2頭は死亡した．剖検で，肝，膵等の諸臓器に著変を認めなかつた．

5)　phospholipase A の種々の量を，犬の主膵管内に，sodium deoxycholate とともに注入せるところ，注入量に応じて種々の程度の膵炎の発生せることが認められた．

以上，急性膵炎に際して膵に活性亢進せる phospholipase A が，門脈血中に逸脱して肝に流入し，あるいは後腹膜に phospholipase A 活性の高い滲出液が直接浸潤すると，生体は重大な悪影響をこうむるものであると考えられる．また，膵炎発生機序の上でも phospholipase A は大きな役割を果しているものと考えられる．

今回の実験で明らかにされた如く，急性膵炎の病因及び病態生理の上で phospholipase A は trypsin に優るとも劣らぬ重要な役割を演じているものであり，従来 trypsin を中心とせる概念のみにては説明し得なかつたいくつかの問題点に，ある程度の解明を与えるものといえよう．